UNIVERSIDAD COMPLUTENSE DE MADRID
FACULTAD DE CIENCIAS BIOLÓGICAS

TESIS DOCTORAL

Choice and inheritance of the habitat : ecological and evolutionary consequences

Elección y herencia del hábitat : consecuencias ecológicas y evolutivas

MEMORIA PARA OPTAR AL GRADO DE DOCTOR

PRESENTADA POR

Gabriel Munar Delgado

Directores

Wilhelmus Maria Cornelis Edelaar
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This thesis has been financially supported by the Spanish Ministry of Universities (FPU17/06268) to Gabriel Munar Delgado, the Spanish Ministry of Science and Innovation (CGL2013-49460-EXP, CGL2016-79483-P and PID2019-108971GB-I00, and the university Pablo de Olavide (Acción Especial del Plan Propio) to Pim Edelaar.

“Nothing inspires more reverence and awe in me
than an old man who knows how to change his mind”

Santiago Ramón y Cajal - Nobel Prize in Physiology or Medicine

“Sometimes science is more art than science, Morty.
Lot of people don't get that.”

Rick Sánchez. Rick and Morty (S1, E6)

Agradecimientos & Acknowledgements

Ha llegado el momento de dar las gracias y me ha sorprendido con la cantidad de gente que me he cruzado durante todos estos años. Creo que esto se convierte en un momento de introspección en el que hay que repasar todo lo acontecido durante la tesis. Ha sido un camino lleno de cosas buenas y bonitas pero tampoco ha sido un camino fácil. Quizás por ello valoro aún más todas las personitas con las que me he cruzado y que han ayudado de una forma u otra a que esto sea posible.

Como parece de rigor, empezaré con mis directores de tesis.

Gracias, Francisco, por aceptar codirigir mi tesis y ayudarme a venir a la UCM a lograr hacer lo que llevaba persiguiendo durante tres años. Gracias por tu simpatía y estar siempre predispuesto a todo.

A Pim, muchas gracias por todo. Quizás no dije que sí a hacer la tesis contigo hasta la vez número 78 que me lo preguntaste, pero al final no me arrepiento de haberlo hecho (todavía jaja). Las circunstancias no han sido siempre las óptimas, pero tú sí. Gracias por empujarme a los congresos, a los cursos y la estancia (y por cubrir después los que te he pedido yo). Gracias por todas las enseñanzas directas e indirectas que me has brindado. Gracias por el apoyo. Gracias también por guiarme y a la vez dejarme hacer y estar abierto todas mis ideas y sugerencias. Aunque esto parecerá imposible si nos ven juntos uno al lado del otro, nunca me he sentido pequeño trabajando contigo y creo que eso dice mucho.

So, talking about the research stay. Thank you very much Christiaan and Marion for welcoming me in Groningen. It was a pleasure to join your team, Christiaan, and being able to learn your way of work. Exchanging ideas with you was very exciting and enriching. Now there is no choice but to continue doing it to publish our super paper.

Thank you to all the RUG people for making me feel at home from the first moment: Koojse, Bea (y Pol!), Nico, Lisa, Drew, Izzy, Marie and my personal Shakira, Michaela. Hope to meet you all again very soon.

But if I felt somewhere more at home than at RUG was at the Biotoop. Thank you to my dutch (but not dutch, family) there. I still miss your spicy cooking, Farhana, Delip and Arif, and of course getting you all drunk haha. But specially many thanks to the part of the biotoop that was conquered by my ancestors some centuries ago. Gracias, Luciana, por todas las risas. Muchas gracias Valeria y Luis por hacer que en ese semi-sótano en el que oscurecía a las 4 de la tarde hubiese luz durante todo el día.

Volviendo al principio, me toca volver a pasar por Sevilla. A aquel laboratorio con inconfundible olor a papilla para mosca y a ese sótano con inconfundible sonido a diamante mandarín. Gracias, Graciela, por la acogida y guiarme en mi primera inmersión en la burocracia de un proyecto. Gracias, Juanra, por ir abriéndome camino y ser mi doctorando de referencia. Mil gracias, Estrella, por el currazo con los diamantes y por compartir conmigo las alegrías y penas del sótano. Igualmente, mil gracias, Paula y María, por sacar a flote todo el trabajazo. Paula, sigue pisando igual de fuerte, ¡que la siguiente eres tú! Gracias, Alicia y Mireia, por los raticos de desconexión. Y muchas gracias, Virginia. Tu llegada al lab, los desayunos y tu compañía hicieron todo más fácil.

Luego tocó la Complu y tres añitos de campo en el Encinar de San Pedro por los que al final ha pasado mucha mucha gente. Gracias a todxs lxs estudiantes de grado y máster que he tenido el placer de codirigir y han hecho una labor titánica en el campo: Laura, Adán, Javier, Adrián, Jessica y Laura (2). Gracias, Chechu y Eva, por toda la inmensa ayuda con la gestión de toda la burocracia y enseñarme a hacer las cosas bien. Mil gracias, Irene, por toda la ayuda y las enseñanzas en el campo anillando bichos. Tu compañía y charlas hacían que levantarse a horas intempestivas fuese fácil. Y gracias al Padrino y a David que también ayudaron. Pero el que se lleva el premio gordo aquí es Alvar. Vaya suerte haberte tenido como técnico de campo. Muchísimas gracias por todo tu curro y siempre de las mejores formas.

Aprovecho para agradecer a toda la gente de Casa de Campo (dirección y mantenimiento) por su buen hacer y ayuda técnica durante el experimento.

Aprovecharé para hacer otro agradecimiento exprés. Muchas gracias a toda la gente bonita que he conocido de congreso, curso (y de festi!) que le ha dado 1000 puntos extra a esta profesión y han sido un enorme incentivo para seguir con esto. Toca destacar a toda la gente del Museo con la que me he cruzado tanto por el Estado como en el extranjero y que me han hecho un huequito y acogido como uno más cuando me ha tocado volar solo. Ojalá nos sigamos cruzando. Hay demasiada gente para nombrar aquí, pero, Jorge, tú te mereces una mención especial. Si seguimos en esto, estoy seguro de que en 20 años nos seguiremos cruzando en todas partes. Eres referente y ha sido/es un honor recorrer este camino de forma paralela contigo y compartir tan buenos ratos. Siguiendo con el Museo, muchas gracias también a Annie y a todo su equipo por su currazo con las muestras de los molineros.

Llegó el plato fuerte, con el despacho 9.4 y sus allegadxs. Gracias a mis mayores Antón, Amparo, Javi, Elena y Dani. Con la mayoría ya me he cruzado más fuera del despacho que dentro y espero que siga siendo así y me sigáis aportando tanto. Muchas gracias, Lucía, has estado ahí para mí incluso desde antes que llegase a la Complu. Aunque tú nos llames a todos pollitos, incluyéndote a ti, creo que tú has sido la mamá pájaro y has cuidado de todxs. Si yo he podido con esto, no tengo ni la más mínima duda de que tú también podrás y aunque ahora esté oscuro, vas a acabar con una tesis tan brillante como tú. Pablo, muchas gracias por todo. Dudo que por este despacho haya pasado una mejor persona que tú. Has conseguido mantenernos a todxs a flote gracias a tu manera de ser (además de que tu coche ha sido excelente para transportar sacos de semillas, pero eso, meh jaja). David, eres ejemplo de perseverancia y superación. Gracias por insistir y que sigamos teniéndote a ti. Gracias por tu empatía y tus halagos. Tienes un potencial más mil veces más grande del que te imaginas. Muchas gracias, Héctor por todos los raticos compartidos y sobre todo por la paliza burocrática final. ¡Mientras escribo esto estás a punto de defender! Espero que la ecología evolutiva nos siga brindando cosas bonitas que podamos compartir. Itziar, ha sido cortito, pero muchas gracias por todo. Nos diste las gracias al llegar, pero también nos tocaba a nosotrxs dártelas a ti. Podrías competir contra Pablo en persona bonita y además tú repartes muchos más abrazos. Mercè, axolote o barbarie. Toda esta gente tiene un potencial enorme, pero creo que todxs coincidiremos en que, si alguien va a llegar lejos en la 100cia, esa vas a ser tú. Gracias por iluminarme con tu perseverancia y por todos los desahogos. Gracias, Marta, por tu simpatía y bondad y hacer del despacho un lugar más bonito. Te mereces toda la suerte del mundo con lo que te esté por llegar. Gracias, Guille, por deleitar con tu pasión por lo bichos y el trabajo de campo. Es un placer compartir ciencia contigo. Muchas gracias también a los anexados Javi, Pablo Yeste y Pedro por lo buenos raticos compartidos juntos. Estoy seguro de que caerán muchos más. Muchas gracias Carmen (eco) por tu contagiosa alegría, vitalidad y ganas de todo.

Ha sido genial cruzarme contigo y estoy seguro que los siguientes momentos van a ser aún mejores. Y muchas gracias al anexo más alejado del despacho, Carmen (micro), por todo el apoyo y todos los ratitos de desconexión que me has dado.

Muchas gracias a mi familia y amigxs. De nuevo, demasiada gente como para dar nombres. Gracias familia por el apoyo desde pequeñito. Un día dije "de mayor quiero ser biólogo" y no me faltó la ayuda ni apoyo en ningún momento. Si algo tienen en un común estos dos grupos es que ninguno sabía (ni sabe) muy bien lo hacía durante la tesis. Y aún así, la frase que más se ha repetido durante estos cuatro años en ambos ha sido: "¿Qué tal están tus pajaritos?" ("Fan bonda es teus pajaritos?"). Muchísimas gracias a todxs. Gracias a mis amigxs porque sin vosotrxs no podría haber completado todo esto.

Por último, tengo que agradecer a Marina todo. Me acompañaste durante la mitad de la tesis y te comiste lo por de ella. Las cosas "pequeñitas" como los madrugones a las 4:30 de la mañana para ir al campo, los 20 ensayos de cada charla, las 56.8763 veces que fallaban los comederos… Pero también las gordas, el estrés, la frustración y la ansiedad. Aun así, siempre respondiste a todo con apoyo y cariño. Gracias a todas las cosas bonitas que compartimos la tesis fue muchísimo más llevadera y conseguí poder con ella.

Muchas gracias a todxs por estar ahí.

Index

Abstract & Resumen

Abstract

Evolutionary ecology could be defined as the study of the adaptation of organisms to environmental variation. This is because generally it is assumed that the causal relationship of adaptation is unidirectional: the environment affects the adaptation of organisms, but organisms do not affect the environment or their adaptedness. At the same time, it is widely recognized that mechanisms that change the environment an individual experiences, such as habitat choice, can enhance the fitness of individuals. This raises the possibility that the causal relationship of adaptation can be bidirectional and means that environmental variation could adapt to organisms. This mechanism of adaptation has been largely neglected in the scientific literature, and its prevalence in nature, and potential ecological and evolutionary consequences are relatively unknown.

In order to understand how organisms can affect the environment to improve their adaptation, the aims of this thesis are: (1) to test how ignoring the adaptation bidirectionality affects estimates of heritability of phenotypic traits when using quantitative genetic animal models; (2) to test whether animal models can be used to estimate the heritability of the environment that individuals experience and how it can evolve, and to investigate whether animal models can estimate the effect of individuals on the environment they experience; (3) to experimentally test the ecological and evolutionary consequences of a type of habitat choice called matching habitat choice, where habitat choice depends on the phenotype of the individual.

We observed that ignoring the fact that individuals (or their phenotype) can affect the environment they experience can result in large biases in the estimates of heritability of

phenotypic traits, or their interpretation. If animal models do not have an adequate causal structure, a false heritability can be detected, and the causal directionality between environment and phenotype can be confused. We also found that animal models are capable of estimating the heritability of the environment (in theory, and in practice) and of determining the effect of an individuals' phenotypic traits on the environment they experience. Therefore, they could be used as a tool to investigate how the environment that individuals experience evolves, which will help to better understand evolutionary dynamics.

Finally, we observed how matching habitat choice can lead to adaptive ecological divergence of populations without current natural selection acting on the ecological trait. Individuals were able to assess their local performance in different local habitats and then settle where it was higher. This spatial self-sorting of individuals indirectly led to assortative mating based on local performance, resulting in reproductive isolation among groups of individuals which differ in local performance. Local adaptation is transmitted to the next generation if offspring is produced within the chosen habitat. These characteristics highlight the potential significance of matching habitat choice in adaptation and evolutionary dynamics. Therefore, future studies should focus on estimating the prevalence of matching habitat choice and its importance in nature.

Resumen

La ecología evolutiva podría definirse como el estudio de la adaptación de los organismos a la variación ambiental. Esto es así porque generalmente se asume que la relación causal de la adaptación es unidireccional: el ambiente afecta a la adaptación de los organismos pero no los organismos no afectan al ambiente o a su propia adaptabilidad. Al mismo tiempo, es reconocido, de forma generalizada, que los mecanismos provocan un cambio en el ambiente que un individuo experimenta, como la selección de hábitat, pueden aumentar el fitness de los individuos. Esto plantea la posibilidad de que la relación causal de la adaptación, en realidad, puede ser bidireccional y la variación ambiental puede adaptarse a los organismos. Este mecanismo de adaptación ha sido ampliamente ignorado en la literatura científica y, por lo tanto, su prevalencia en la naturaleza y sus potenciales consecuencias ecológicas y evolutivas son relativamente desconocidas.

Para comprender cómo los organismos afectan al ambiente para mejorar su adaptación los objetivos de esta tesis son: (1) testar cómo ignorar la bidireccionalidad de la adaptación afecta a las estimas de heredabilidad de los rasgos fenotípicos cuando se usan animal models de genética cuantitativa; (2) testar si los animal models pueden ser usados para estimar la heredabilidad del ambiente que los individuos experimentan y cómo este puede evolucionar e investigar si los animal models pueden estimar el efecto de los individuos sobre el ambiente que experimentan; (3) testar experimentalmente las consecuencias ecológicas y evolutivas de un tipo de hábitat denominado elección de hábitat coincidente en el cual la elección de hábitat depende del fenotipo del propio individuo.

Observamos que ignorar el hecho de que los individuos (o su fenotipo) pueden afectar al ambiente que ellos mismos experimentan puede resultar en un amplio sesgo de las estimas de heredabilidad de los caracteres fenotípicos o de interpretación de los mismos. Si los animal models no tienen la estructura causal adecuada, se puede detectar una heredabilidad falsa y la direccionalidad causal entre el ambiente y el fenotipo puede ser malinterpretada. También demostramos que los animal models son capaces de estimar la heredabilidad del ambiente (en la teoría y en la práctica) y determinar el efecto del fenotipo de los individuos en el ambiente que ellos experimentan. Por lo tanto, pueden ser usados como una herramienta para investigar cómo el ambiente que los individuos experimentan evoluciona, lo que ayudará a comprender de una manera más precisa las dinámicas evolutivas de las poblaciones.

Finalmente, observamos cómo la elección de hábitat coincidente puede provocar divergencia ecológica de poblaciones adaptativa sin que la selección natural actúe sobre el rasgo ecológico. Los individuos fueron capaces de evaluar su rendimiento en diferentes ambientes locales y luego asentarse dónde era mayor. Esta auto-separación ordenada de los individuos indirectamente provocó emparejamiento concordante basado en el rendimiento local, lo que resultó en aislamiento reproductivo entre los grupos de individuos que diferían en rendimiento local. Por último, si la descendencia se produce dentro del hábitat elegido, entonces la adaptación local se transmite a la siguiente generación. Estas características resaltan la potencial relevancia de la elección de hábitat coincidente en el proceso de adaptación y en las dinámicas evolutivas. Por l tanto, futuros estudios deberían enfocarse en estimar la prevalencia e importancia del mecanismo de elección de hábitat coincidente en la naturaleza.

Introduction

Introduction

The Anthropocene and the adaptation challenge

In the era of the Anthropocene, humans have become the most dominant force in reshaping the planet (Waters et al., 2016). The widespread impact of human activities on the environment has led to unprecedented global changes of the Earth's ecosystems, including habitat destruction and climate change. In this context, organisms are bound to face novel and rapid ecological challenges at a rate that often overcomes their adaptive capacity, leading to population declines and, ultimately, extinction (Thomas et al., 2004). Many populations struggle to cope with these ecological challenges and to adapt to environments suddenly disrupted by humans. For this reason, one of the biggest current concerns in evolutionary ecology is understanding how populations adapt to the environment, which might allow us to mitigate the effects of human activities on biodiversity loss.

Different routes towards adaptation

Adaptation is the key process by which organisms cope with changes in their environment. Individuals interact with different local environments, and through the process of local adaptation, they maximize their fitness in the face of any trade-offs (Hereford, 2009; Kawecki & Ebert, 2004). In this way, adaptation essentially causes a match between environmental conditions and an organism's ecological traits (Edelaar & Bolnick, 2019).

The most well-known mechanism driving local adaptation is natural selection. When there is variation among individuals in their phenotype-environment match, those individuals with a better match achieve a higher reproductive rate. If this match is heritable, the number of

individuals with a better match increase, while those with a worse match (= greater mismatch) decline in the population. This results in a better adapted population, or, in other words, in adaptive evolution of the population.

Often, natural selection is viewed as the sole mechanism for driving population adaptation (Futuyma, 2017; Schluter, 2001), but other mechanisms can also contribute to this. Edelaar and Bolnick (2019) proposed a framework that classifies alternative and/or complementary routes to adaptation, depending on whether changes occur in the phenotype or in the environment, and whether they occur through selection or through alteration. They thereby identified four mechanisms that can increase fitness based on the phenotype-environment match: natural selection, change of the phenotype, adjustment of the environment, and selection of the environment (Fig. 1). While natural selection can only increase adaptation or phenotype-environment match at the population level, the remaining three mechanisms operate at the individual level, increasing individual adaptation, which may then have an impact on adaptation at the population level. Understanding the relevance of these individual-level mechanisms, particularly in the context of rapid global change, could improve biodiversity management and mitigate negative effects of global change. However, despite their potential importance, some of these mechanisms have been relatively overlooked in evolutionary ecology, due to the focus on natural selection rather than on the increase of the phenotype-environment match (Edelaar et al., 2023).

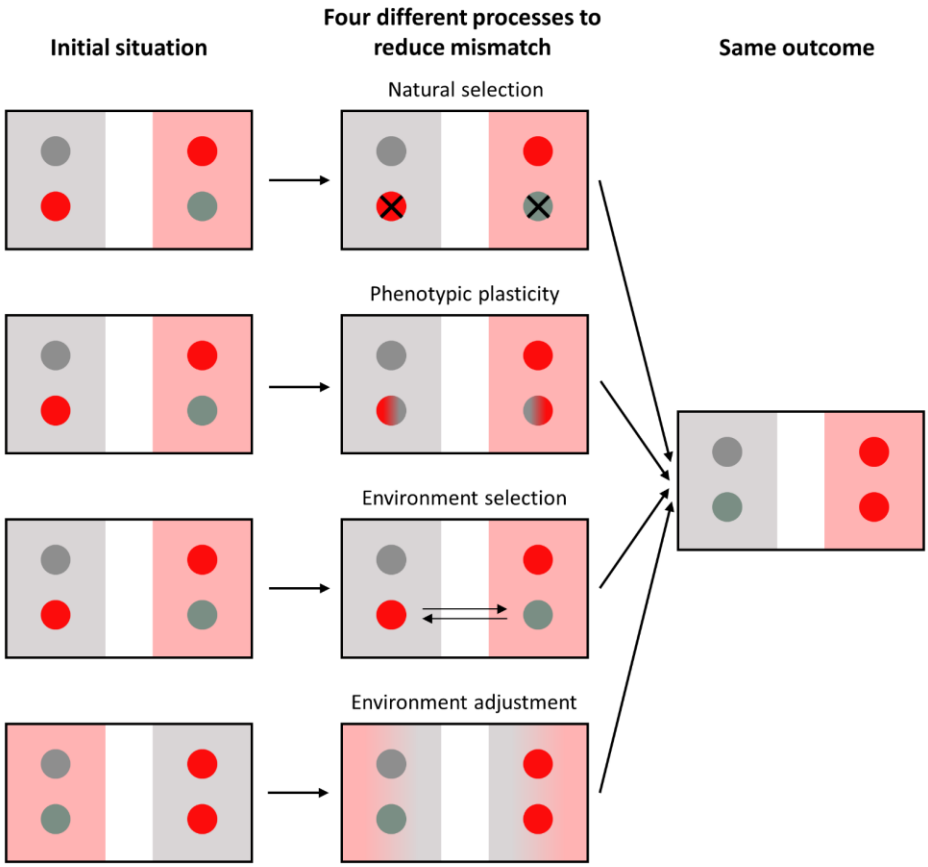

Figure 1. The four adaptation processes highlighted by Edelaar and Bolnick (2019). Each box represents individuals in two different separated environments. Individuals are represented by colored dots and local environments by colored backgrounds. When colors match, there is a match between their phenotype and their local environment, resulting in adaptation to that environment. All four processes, namely natural selection, phenotypic plasticity, environment selection, and environment adjustment, can improve initial individual mismatch and ultimately lead to the same outcome of a matching pattern.

Selection of the environment as a mechanism of adaptation

One of the main reasons why selection of the environment has been relatively neglected in the literature on adaptation is because it implies individual dispersal movement. And, traditionally, dispersal has been considered as a mechanism counteracting natural selection and population

adaptation (Lenormand, 2002; Smadja & Butlin, 2011). While natural selection promotes local adaptation by increasing the frequency of locally adapted phenotypes, and, as a consequence, of adapted genotypes, movement of individuals between locally adapted populations has been thought to potentially introduce maladaptive phenotypes and genotypes to those populations (Aitken & Whitlock, 2013; Fitzpatrick & Reid, 2019). Thus, it has been widely assumed that gene flow between populations reduces phenotype-environment match, and, thereby, constrains local adaptation (Postma & van Noordwijk, 2005). However, this may not hold true if gene-flow between different environments and populations is not random.

To illustrate how non-random gene flow can improve individual fitness, here, I will focus on habitat choice as a form of selection of the environment (broader sense; Edelaar & Bolnick, 2019). Specifically, I will focus on the three types of habitat choice distinguished by Ravigné et al. (2004) and Akcali & Porter (2017): plastic habitat choice, direct genetic habitat choice and matching habitat choice.

Plastic habitat choice encompasses habitat choice that is driven by an individual preference acquired during its development in response to an environmental cue (e.g., Stamps et al., 2009). It includes “learned habitat choice”, “habitat imprinting” and “natal habitat preference induction”. This kind of habitat choice can be adaptive, for example, when genotypes differentially reproduce in different habitats and offspring are imprinted on their natal habitat. Then, offspring would choose habitats similar to those where they were born, and to which they are potentially better adapted due to their genetic resemblance to their parents: what worked for their parents probably works for them too.

Direct genetic habitat choice encompasses habitat choice that is directly induced by preference alleles for a specific habitat. Note that although all habitat choice mechanisms ultimately involve genes, in this mechanism, habitat alleles directly cause preference for specific habitats (e.g., alleles changing taste receptors in phytophagous insects, thereby causing preferences for specific host plants; Matsubayashi et al., 2010). When such preference alleles differ among individuals and are coupled with other phenotypic differences that increase the match with the preferred habitat, direct genetic habitat choice is adaptive.

Matching habitat choice encompasses performance-dependent habitat choice (Edelaar et al., 2008). Here individuals assess their local performance across different habitats and, based on this, settle where their ecological performance is highest, i.e., where the phenotype-environment match is maximized. This kind of habitat choice is therefore adaptive by definition, at least for the traits involved in the assessed performance.

Thus, all three different forms of habitat choice can cause adaptive gene flow, because the movement of individuals is not random but towards habitats that provide greater expected fitness.

The neglected ecological and evolutionary consequences of matching habitat choice

The adaptive components of plastic and direct genetic habitat choice rely on the past match between phenotypes and local environments: the preference predicts fitness if traits and environments have not changed between generations. We can assume that natural selection favored the appearance of these mechanisms because they improve environment-phenotype match. However, while these preferences (due to imprinting or preference alleles) are somehow fixed for the organism (Davis & Stamps, 2004), the traits or the environment may change. If

the genetic preferences of an individual become decoupled from the phenotypic traits that determine fitness (due to factors such as mutation, or recombination), habitat preference may become maladaptive (Remeš, 2000). The same could occur for plastic habitat choice, if offspring phenotypes differ from their parents' phenotypes in a way that does not match their natal habitat. Alternatively, it could happen that environmental cues remain equal but other environmental aspects that influence ecological performance change, causing ecological traps. Genetic preference and imprinting may not be able to deal with this, and lead to maladaptation. In contrast, matching habitat choice (MHC) does not rely on past static "expectations" for phenotype-habitat match. Instead, individuals can continually assess their own performance across different and dynamically changing local habitats and choose a habitat based on their present ecological performance, depending on the match of their current ecological traits with the current environment. This implies that under MHC, gene flow would always be adaptive (Edelaar & Bolnick, 2012). Maladapted individuals in one habitat would move to better matching habitats that increase their adaptation, which would, in turn, increase overall adaptation of both the donor and receptor populations. Thus, the inherent ecological result of MHC is a spatial self-sorting of individuals with different locally adapted phenotypes clustering in different habitats, or, in other words, ecological population divergence (Edelaar et al., 2008; Edelaar et al., 2019; Camacho et al., 2020). This is the same adaptive output that would be expected under the action of divergent natural selection (Fig. 1), but in this case, without the need for the existence of differential reproductive success or survival.

MHC, consequently, promotes covariance between phenotypic traits and local environments (Edelaar & Bolnick, 2012). Based on theoretical work, this is thought to have several potentially relevant evolutionary implications, which, to date, have hardly been tested experimentally. First, when individuals with different phenotypic characteristics move to

different local habitats, they are also expected to mate there. Thus, MHC is thought to indirectly promote assortative mating, with individuals mating with other individuals with a similar phenotype and local performance (Edelaar et al., 2008; Porter & Benkman, 2022). Second, if individuals with similar phenotypes mate with each other in different habitats, then reproductive isolation between individuals with distinct phenotypes is expected to emerge (Edelaar et al., 2008; Porter & Benkman, 2022). Third, offspring of assortative pairs are expected to have a phenotype similar to their parent's phenotypes. If this offspring is born in the matching habitat chosen by their parents and where they mated, offspring phenotype is expected to match with their natal habitat. Following this causal path, the adaptive ecological population divergence driven by MHC within a generation can be inherited by the next generation, making MHC a driver of adaptive evolution.

Unfortunately, while MHC has the potential for yielding significant adaptive and evolutionary outcomes, its study has been very limited, resulting in a lack of experimental support for its evolutionary implications beyond promoting ecological divergence.

Chosen habitat as a heritable extended phenotype

Dawkins (1982) referred to the term extended phenotype as "the long reach of genes," to the expression of the genotype in the external environment. Consequently, the extended phenotype has typically been understood as the adjustments individuals make to their environment. When these adjustments impact an individual's own fitness, the extended phenotype can evolve via natural selection, like any other phenotypic trait (Dawkins, 1982). For instance, when a bird's nest affects its reproductive success, natural selection favors nests that increase reproductive success, provided that the nest (or the capacity to build it) is inherited, at least to some extent. However, Edelaar and Bolnick (2019) expanded the concept of the extended phenotype to also

include the selected environment. In this context, when a bird selects a nest site (such as a nest cavity), and its characteristics affect reproductive success, the outcome is similar to that of nest building, and the chosen nest site can be considered an extended phenotype. Here I use this more generalized definition of the term, and treat every local habitat that depends on the individual and that can potentially affect its local adaptation (i.e., local fitness), as an extended phenotype. Consequently, it includes the local environment selected by either plastic habitat choice, direct genetic habitat choice, or matching habitat choice.

As outlined above, habitat choice can improve the environment-phenotype match and increase individual fitness. Disentangling how phenotypes evolve to improve this match is considered essential for fully understanding the adaptation process (Schluter, 2001). In the same way, it is crucial to disentangle how the local environment an individual experiences evolves (Edelaar et al., 2023; Laland et al., 2016). Considering the chosen habitat as an extended phenotype, i.e., as an integral part of an organism with its own heritable basis and evolutionary potential, could facilitate a better understanding of the environment-phenotype match and the local adaptation process. Although theoretical work on how individuals inherit the extended phenotype and its evolutionary consequences has been done (Odling-Smee et al., 2003, Laland et al., 2019; Bolnick & Otto, 2013; Edelaar & Bolnick, 2012), there are relatively few studies that have estimated its heritability in wild populations. As a result, the link between theory and reality is weak. Emphasizing the evolution of the extended phenotype as a form of population adaptation and providing practical tools to test this process in natural populations should increase research interest in this promising yet neglected area.

A multi-pronged approach to shed light on a complex and (relatively) unexplored field

As outlined above, there is a significant gap in our knowledge of how habitat inheritance and habitat choice contribute to population adaptation. In this PhD thesis, I use a multi-pronged approach to contribute to fill this gap.

The thesis is divided into two different blocks. In the first block, I characterize the inheritance of habitat as an extended phenotype, explore its evolutionary consequences, and provide practical tools for its study. In the second block, I experimentally test the ecological and adaptive evolutionary consequences of matching habitat choice. Each of these two blocks is divided into two chapters, in which I focus on different subjects using different approaches, as follows.

In **Chapter I,** I characterize the local environment as an extended phenotype. We argue that since it depends on an organism and it is modified by selection, it is justified to be theoretically and statistically treated as an organismal trait. We then simulate different biological scenarios in which a virtual population has a genetic basis for a focal phenotypic trait and/or an extended phenotype. We also simulate an effect of the environment on the focal phenotypic trait, and vice versa. The first is the already well-known phenotypic plasticity, and for the second we introduce a novel term which we call 'environmental plasticity'; it encompasses the effect of the phenotype on the environment (its selection or adjustment). We then use animal models, a statistical tool from the field of quantitative genetics traditionally used to estimate the heritability of phenotypic traits, to assess the consequences for the output of these models when the environment is considered an extended phenotype.

In **Chapter II,** I apply the lessons learnt from Chapter I to a real population. I use an extensive dataset from a long-term population study on pied flycatchers that includes information on the environment of each individual: the composition of the vegetation around their nest box. We first treat the vegetation composition as an extended phenotype to estimate its heritability. We then take it a step further and attempt to elucidate the mechanism responsible for the observed inheritance of the extended phenotype. By simulating various scenarios where I randomize the nest box location, I determine the mechanism responsible for habitat inheritance.

In **Chapter III,** I focus on experimentally testing the adaptive and evolutionary consequences of MHC. To do this, we use a captive population of zebra finches. To encourage the emergence of MHC in our study population, we modified the local performance of the birds at the extremes of an aviary. For this, we equipped our zebra finches with a plastic ring with a transponder tag that allowed them to access the food in specific, programmable electronic feeders. We placed half of the feeders in one extreme of the aviary and the other half in the other extreme. Then, we programmed the feeders at one extreme to only give access to food to half of the zebra finches, while the feeders at the other extreme gave access to the other half of the individuals. Thus, we generated maximum variation in local performance in our zebra finch population. We provided nest boxes at each extreme of the aviary and allowed the birds to breed. In this experiment, we observe how MHC emerged and experimentally confirm some of the expected ecological and evolutionary outcomes.

Finally, in **Chapter IV,** we use a similar experimental system to the one in Chapter III, but this time we apply it to a population of tree sparrows in the field. We placed electronic feeders in two different areas in the field that were also provided with nest boxes. We set the feeders in one area to give access to supplementary food to half of the population of ringed tree sparrows,

and the feeders in the other area some 300 meters away to give access to the other half. We additionally equipped the nest boxes with electronic readers to verify the identity of the breeding tree sparrows. We again observe how MHC emerged, and obtain experimental support for the adaptive evolutionary consequences of MHC.

Chapter I: Estimation of additive genetic variance when there are gene–environment correlations: pitfalls, solutions and unexplored questions

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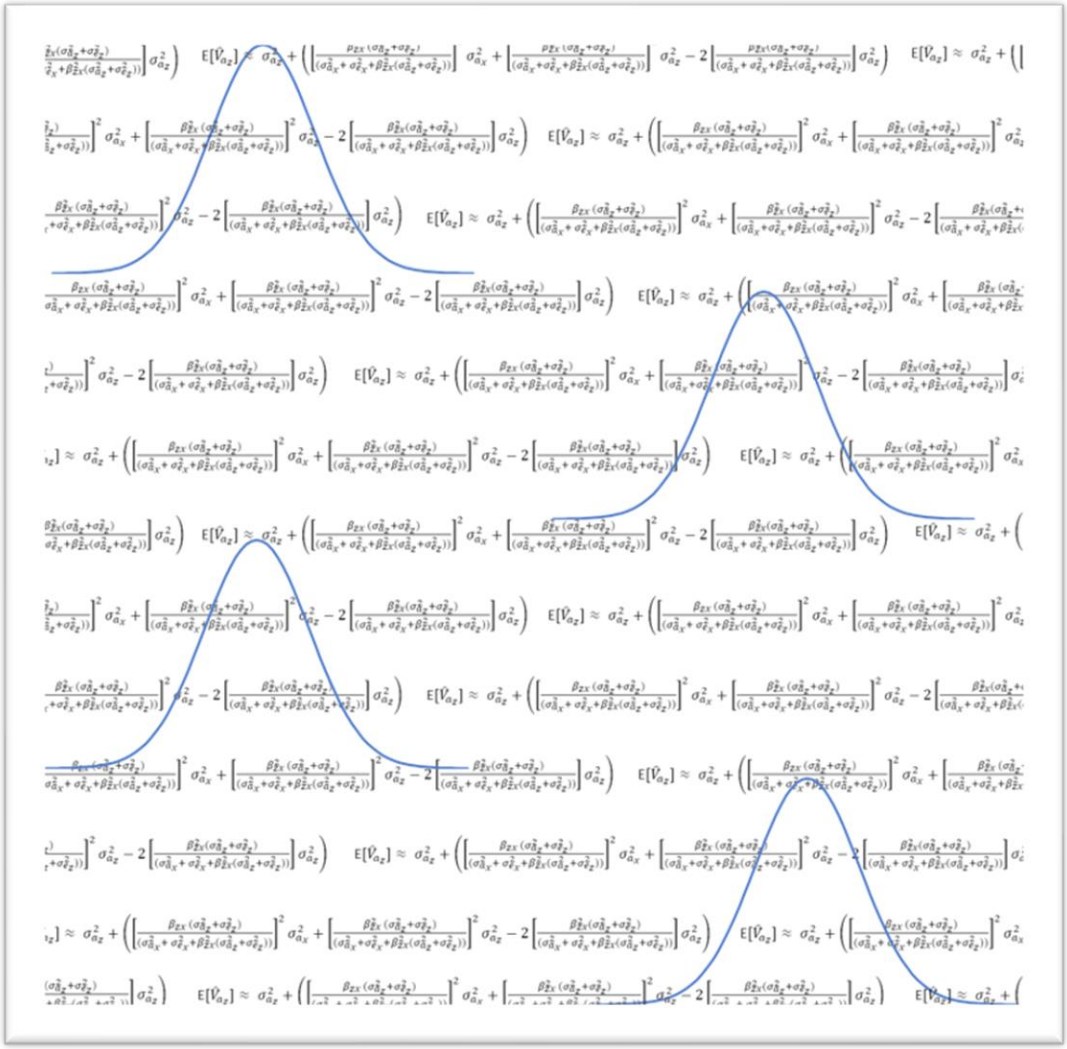

This chapter reproduces entirely the published article:

Munar-Delgado, G., Araya-Ajoy, Y. G., & Edelaar, P. (2023). Estimation of additive genetic variance when there are gene–environment correlations: Pitfalls, solutions and unexplored questions. *Methods in Ecology and Evolution*, 00, 1– 14. https://doi.org/10.1111/2041-210X.14098

Abstract

Estimating the genetic variation underpinning a trait is crucial to understanding and predicting its evolution. A key statistical tool to estimate this variation is the *animal model*. Typically, the environment is modeled as an external variable independent of the organism, affecting the focal phenotypic trait via phenotypic plasticity. We studied what happens if the environment is not independent of the organism because it chooses or adjusts its environment, potentially creating non-zero genotype-environment correlations. We simulated a set of biological scenarios assuming the presence or absence of a genetic basis for a focal phenotypic trait and/or the focal environment (treated as an extended phenotype), as well as phenotypic plasticity (the effect of the environment on the phenotypic trait) and/or 'environmental plasticity' (the effect of the phenotypic trait on the local environment). We then estimated the additive genetic variance of the phenotypic trait and/or the environment by applying 5 animal models which differed in which variables were fitted as the dependent variable and which covariates were included. We show that animal models can estimate the additive genetic variance of the local environment (i.e., the extended phenotype) and can detect environmental plasticity. We show that when the focal environment has a genetic basis, the additive genetic variance of a phenotypic trait increases if there is phenotypic plasticity. We also show that phenotypic plasticity can be mistakenly inferred to exist when it is actually absent and instead environmental plasticity is present. When the causal relationship between the phenotype and the environment is misunderstood, it can lead to severe misinterpretation of the genetic parameters, including finding "phantom" genetic variation for traits that, in reality, have none. We also demonstrate how using bivariate models can partly alleviate these issues. Finally, we provide the mathematical equations describing the expected estimated values. This study highlights that not taking gene-environment correlations into account can lead to erroneous interpretations of additive genetic variation and phenotypic plasticity estimates. If we aim to understand and

predict how organisms adapt to environmental change, we need a better understanding of the mechanisms that may lead to gene-environment correlations.

Key words: additive genetic variance, animal model, bivariate model, environmental plasticity, extended phenotype, gene-environment correlation, gene-environment covariance, phenotypic plasticity

Introduction

One of the biggest current challenges in evolutionary biology is understanding how populations adapt to their environment and predicting if they will be able to cope with the pace of anthropogenically induced environmental change. It is thus essential to understand the genetic and phenotypic changes that allow populations to cope with environmental challenges (Chevin et al., 2010). Within this context, the field of quantitative genetics studies the additive genetic variance of traits. This is a key determinant of their heritability and evolutionary potential and, thus, of the ability of populations to adapt to their environment (Falconer & Mackay, 1996). One of the most widely used statistical tools for estimating the additive genetic variance of a trait is a type of mixed model called the "animal model" (Lynch & Walsh, 1998; (Kruuk, 2004).

Various studies have highlighted possible pitfalls when using animal models, and the errors associated with their application and biological interpretation (de Villemereuil et al., 2018; Kruuk, 2004; Kruuk et al., 2008; Postma & Charmantier, 2007; Wilson et al., 2010). For example, failing to account for maternal effects may cause additive genetic variance (and thus heritability) to be overestimated due to the genetic covariance between siblings as generated by the shared environment (e.g., the care of their mother) (Wilson et al., 2010).

Another problem with the animal model is that the assumed model structure might not reflect the actual biology of the system (Westneat et al., 2020), and this has received far less attention. Here we specifically focus on the assumption that genes and environment are uncorrelated (Lynch & Walsh, 1998) (Fig. 1, a). If not, the gene-environment covariance changes the estimated phenotypic variance:

$$V_P = V_G + V_E + 2Cov[G,E] \text{ (1).}$$

Where V_P is the phenotypic trait variance, V_G is its additive genetic variance, V_E its environmental variance and $Cov[G,E]$ is the gene-environment covariance. That this covariance is zero (i.e., that genotypes are randomly distributed across environments) may be true in captivity or other controlled conditions, but not necessarily so in natural populations. Although this problem has long been recognized (e.g., Falconer, 1960), it seems to be systematically ignored (e.g., it is not mentioned in Charmantier et al., 2014).

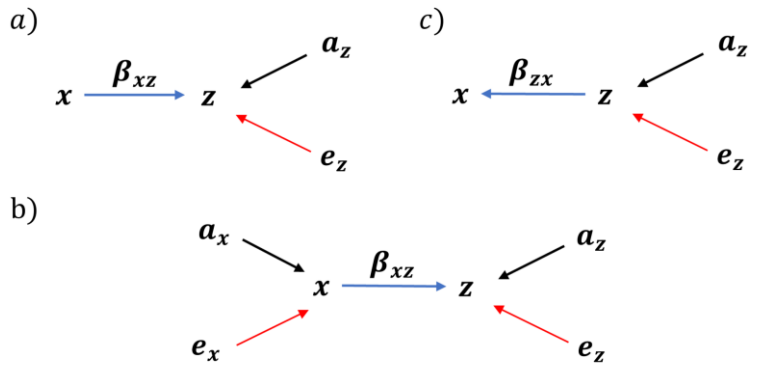

Figure 1. a) Typical quantitative genetic partitioning of focal phenotypic trait z into direct additive genetic (a_z), environmental (x) and residual (e_z) components. A known environment may affect the phenotypic trait via phenotypic plasticity (β_{xz})**,** but this effect is independent of the organism's genes. **b)** Compared to a), when an organism has a genetic preference (a_x) to choose or adjust its local environment (x)**,** the genes of the organism and its environment are no longer independent. In this scenario the genes influencing choice or adjustment of the local environment also indirectly influence the phenotypic trait through phenotypic plasticity (β_{xz}). **c)** When the phenotypic trait (z) of an organism has a genetic component (a_z) and affects the choice or adjustment of the local environment (x) via "environmental plasticity" (β_{zx}), the genes underpinning the expression of the phenotypic trait indirectly affect the choice or

adjustment of the environment. Thus, the genes of the organism and its environment are no longer independent, causing a genetic correlation between the organism and its environment.

A common approach to deal with phenotype-environment relationships is to fit the environment as a covariate. However, this approach implicitly assumes that their covariance is environmental instead of genetic. This assumption may be incorrect in natural populations for two reasons. First, there might be a genetic basis to aspects of the environment that we include in the model (Fig. 1, b). This could occur when the organism has a (genetic) preference to occur in a specific type of environment ("selection (choice) of the environment" cf. Edelaar & Bolnick, 2019, e.g., habitat choice), or when it has a (genetic) inclination to change its environment to a different state ("adjustment of the environment" cf. Edelaar & Bolnick, 2019, for example habitat construction). Although it has been shown that genetic variation can affect and determine an individual's environment (e.g., Dawkins, 1982; (Jaenike & Holt, 1991; Weber et al., 2013), this possibility is rarely explored using animal models (see Järvinen et al., 2017; Gervais et al., 2020 and Gervais et al., 2022 for exceptions).

A second case that violates the assumption that the organism and the environment are independent occurs when a phenotypic trait influences how the local environment is adjusted or which environment is chosen (e.g., phenotype-dependent matching habitat choice, Edelaar et al., 2008) (Fig. 1, c). This is an effect of the phenotypic trait on the focal environment, the reverse effect of phenotypic plasticity (where the environment affects the phenotypic trait). For lack of an established term (as far as we know), we will call this "environmental plasticity" as a logical equivalent of phenotypic plasticity. Environmental plasticity covers the phenotype-dependency of both choice and adjustment of the local environment that an individual

experiences. Note that for habitat choice/selection, for an outside observer the environment does not undergo any transformation. However, the observing researcher is irrelevant: the local environment that the individual organism experiences does indeed change, and this is what matters. In this way, if an organism's focal phenotypic trait harbors genetic variation and affects the choice or adjustment of its local environment (i.e., there is environmental plasticity), then a genetic covariance between the trait and the environment is expected. Assuming the inverse causal relationship of an effect of the focal environment on the phenotypic trait (i.e., assuming phenotypic plasticity when there is environmental plasticity) could cause wrong inferences of the animal model estimates. As an example, imagine we are studying the heritability or plasticity of behavioral boldness of a breeding wild bird population. We might be tempted to add nest distance from the closest road as a fixed effect to control for the influence of human disturbance on boldness (i.e., phenotypic plasticity). Alternatively, boldness could affect nest distance choice (i.e., environmental plasticity, with less bold individuals preferring to breed further away from roads) instead of the other way around (Holtmann et al., 2017) . For this scenario, treating boldness as a response variable and nest distance as the independent variable would not reflect the true causal relationship. This could result in misinterpretation of the estimates provided by the animal model due to the misspecification of the causal structure of the model.

When studying gene-environment correlations, it is important to emphasize that for any hypothesized relationship between phenotypes and environment, the estimated genetic variance can be underpinned by "direct" genetic variance affecting a trait or environment versus "indirect" genetic variance. We therefore use the term "direct additive genetic variance" to refer to the variance caused by alleles "directly" affecting a trait (path a_z to z in Fig. 1, a), in the sense that the causally intermediate traits are not measured or of interest. Within a path

analysis context this has been referred to as exogenous variance because factors outside the causal pathway cause it (e.g., Villemereuil et al., 2018). In the path diagram depicted in Fig. 1 a, the direct variance can also be thought of as the expected genetic variance on the trait conditional on all individuals having the same focal environment. In contrast, we use the term "indirect additive genetic variance" to acknowledge that alleles underpinning traits affecting the environment may cause indirect genetic variance on other traits, because of the indirect effects of alleles on a phenotype through the environment (path a_x to x to z in Fig. 1, b). In other words, variance that is caused by a plastic response to variation in a phenotype or environment with a genetic underpinning. Finally, we use the term "total additive genetic variance" to refer to the sum of both direct and indirect genetic variance.

When the local environment varies depending on the individual's genotype, it may be seen as an extended phenotype, i.e., the expression of genes in traits outside of what is typically considered the organism (Dawkins, 1982; Edelaar & Bolnick, 2019). Studying environmental variables as extended phenotypes allows linking the study of gene-environment correlations to previous treatments on selection on causally covarying traits (e.g., (Morrissey, 2014, p. 200)). Contrary to Dawkins's definition, here we use extended phenotype without implying that the choice or adjustment of the focal environment is expected to result in a change in fitness. (See supplementary materialI for further discussion on treating environmental variables as extended phenotypes).

In this paper, we combine data simulations with mathematical derivations to describe the consequences for the animal model estimates and their interpretation when the focal environment depends on the genes of our study organism. We simulated data assuming 12

different biological scenarios and then analyzed these data with a set of 5 animal models with different causal structures. The fitted animal models varied in which trait was the focal trait and the covariate (including bivariate models). We then show how the additive genetic variance estimated by the different animal models ($\hat{V}_a$) fitted for the different biological scenarios can either reflect a trait's *direct* additive genetic variance, its *indirect* additive genetic variance, the sum of these two (i.e., its *total* additive genetic variance), or a biased estimate which is not consistent with anything of the above. What the animal model provides us with depends on the existence of gene-environment correlation and the appropriateness of the model structure for each biological scenario (see Table S1 for the expected estimated values for each model for each scenario).

Methods

General simulation design

We developed a simulation procedure in *R* 4.0.2 (R Core Team, 2020) to study the impact of gene-environment correlations on the estimates of plasticity and additive genetic variance. This simulation generates an individual phenotypic trait (z) which has a genetic underpinning summarized by a breeding value (α_z), which responds plastically (β_{xz}) to a focal environmental variable (x) and which is affected by unknown effects summarized in a residual value (ε_z),

$$z = \alpha_z + \beta_{xz}x + \varepsilon_z \quad (2).$$

In a similar way, the focal environment variable may have a genetic underpinning summarized by a breeding value (α_x), it can also be affected by the organism's phenotypic trait (z) proportional to the coefficient β_{zx} , and it is also affected by unknown residual effects (ε_x)**,**

$$x = \alpha_x + \beta_{zx}z + \varepsilon_x \quad (3).$$

The unknown residual effects on the phenotypic trait (ε_z) and the environment (ε_x) are assumed to be a realization of a normal distribution with a mean of zero and variance $\sigma^2_{\varepsilon_z}$ and $\sigma^2_{\varepsilon_x}$, respectively.

The breeding values for each individual are the sum of the effects of n loci (in linkage disequilibrium) influencing trait expression. Each locus has two alleles coded as 0 and 1, and for simplicity, we assume that their effects on the trait are additive. Also, for simplicity, we assume that allele frequencies for all loci are 0.5. Therefore, the expected direct additive genetic variance in a trait ($\sigma^2_{\alpha_z}$) or environment ($\sigma^2_{\alpha_x}$) is equal to $0.5^2 n$, where n can differ between the phenotypic trait and the focal environment.

We start by simulating the genotypes of a founder population of 750 individuals; then sexes are randomly assigned ensuring a sex ratio of 1. Individuals mate randomly, there is no natural selection, and there are no overlapping generations. Alleles follow Mendelian segregation, without mutation. We simulate 10 non-overlapping generations and each pair produces 2 individuals, ensuring a constant population size across generations. The genotype of each individual is then used to calculate its breeding value. Finally, pedigrees are built based on the parent-offspring relationships (See supplementary material for more details on the simulation procedure).

Biological scenarios

We simulated 12 different biological scenarios (Table 1) that involved the presence or absence of the following factors: direct additive genetic variance for the phenotypic trait ($\sigma^2_{\alpha_z}$=25),

direct additive genetic variance for the focal environment ($\sigma^2_{\alpha_x}$=12.5), phenotypic plasticity (β_{xz}=0.7) (i.e., the effect of the focal environment on the phenotypic trait, Fig. 1, a-b) and environmental plasticity (β_{zx}=0.5) (i.e., the effect of the phenotypic trait on the focal environment, Fig. 1, c). A fixed variance in *z* and *x* due to unknown residual effects was always simulated (120 and 70 respectively). These values are arbitrary, but we intentionally used values to make the simulation results clear. Every scenario was simulated 100 times, resulting in 100 data sets that differed due to stochastic variation. The simulation results are only valid for the specific choices of each simulation, and serve mostly as examples. Therefore, we generalize the simulation results by providing the analytical formulas for the expected estimated values by the different animal models for each scenario.

	Scenario											
Simulated parameter	1	2	3	4	5	6	7	8	9	10	11	12
$\sigma^2_{\alpha_z}$ **=25**	-	-	✓	✓	-	✓	-	✓	-	-	✓	✓
$\sigma^2_{\alpha_x}$ **=12.5**	-	-	-	-	✓	✓	✓	✓	-	✓	-	✓
β_{xz} **=0.7**	-	✓	-	✓	-	-	✓	✓	-	-	-	-
β_{zx} **=0.5**	-	-	-	-	-	-	-	-	✓	✓	✓	✓

Table 1. Parameters used for simulating in each scenario. "✓" indicates when a particular parameter was involved using the given values, where $\sigma^2_{\alpha_z}$ is direct additive genetic variance for the focal phenotypic trait, $\sigma^2_{\alpha_x}$ is direct additive genetic variance for the focal environment, β_{xz} is phenotypic plasticity, and β_{zx} is environmental plasticity.

Scenarios 1-4 correspond to populations with a phenotypic trait with a direct genetic basis and/or phenotypic plasticity. These scenarios were used to check the simulation procedures since they are the classical scenarios usually assumed when using animal models. In scenarios 5-8, we simulated a direct genetic basis for the focal environment. In scenarios 7 and 8, we also simulated phenotypic plasticity. In these hypothetical scenarios, the focal phenotypic trait is affected by an environmental variable with a direct genetic basis. For example, when the water depth at which a deep-sea fish forages has a direct genetic basis (Gaither et al. 2018) and this depth (focal environment) affects its body mass (phenotypic trait). For scenarios 9-12, we simulated environmental plasticity instead of phenotypic plasticity. In other words, in these scenarios, we simulated that the focal environment was affected by the focal phenotypic trait. One such example is shown in Camacho et al. (2020), where ground-perching grasshoppers of a specific color (focal phenotypic trait) choose a substrate of a color (environmental variable) similar to their own to increase crypsis. This does not change the actual color of any of the available substrates, but it does change the color of the environment that each individual experiences, which is our focal trait. For simplicity, we did not simulate scenarios where phenotypic and environmental plasticity are simultaneously present, as this leads to feedback and possible order effects, although it appears to be possible in nature (Lowe & Addis, 2019; Boyle & Start, 2020).

Statistical analyses: animal models

We fitted the animal models using the R package *ASReml-R* (Butler, 2020). See Table 2 for parameter descriptions.

Parameters	
Simulated	Description
z	Focal phenotypic trait
x	Focal environment/extended phenotype
a_z	Breeding value for the phenotype
e_z	Effect of residual variables on the phenotypic trait
a_x	Breeding value for the focal environment
e_x	Effect of residual variables on the focal environment
$\sigma^2_{a_z}$	Direct additive genetic variance of the phenotypic trait
$\sigma^2_{a_x}$	Direct genetic variance of the focal environment
β_{xz}	Strength of the effect of the focal environment on the phenotypic trait (i.e., phenotypic plasticity)
β_{zx}	Strength of the effect of the phenotypic trait on the focal environment (i.e., environmental plasticity)

Table 2. Notation and description of each of the parameters that were simulated. To distinguish estimated parameters for (co)variances and types of plasticity from simulated ones, we denote parameter estimates using a hat symbol (e.g., $\hat{\beta}_{xz}$). Note that breeding values and residual values are mean centered for equations 4-8, representing the statistical analyses but not for equations 2 and 3, describing the simulation process. We thus represent them with different symbols. We also refer to $\hat{V}_a$ to the estimates of additive genetic variance to highlight that this may or may not represent any of the values used for the simulation.

We fitted five different animal models. Models 1 and 2 correspond to the typical structures usually used to estimate the genetic parameters of the phenotypic trait of interest (*z*). Model 1 is the simplest case:

$$z = u_z + a_z + e_z \ (4).$$

Where u_z is the population mean, a_z are the breeding values with variance $\hat{V}_{a_z}$ and e_z is the residual term with variance $\hat{V}_{e_z}$.

Model 2 has a similar model structure but includes the focal environment of each individual (*x*) as a fixed effect and the effect of such an environment on the individuals' phenotypic trait ($\hat{\beta}_{xz}$):

$$z = u_z + \hat{\beta}_{xz}x + a_z + e_z \ (5).$$

Model 3 is similar to model 1, but now the environment is the trait of interest (the dependent variable), potentially with its own genetic basis:

$$x = u_x + a_x + e_x \ (6).$$

where x is the experienced focal environment variable of each individual, u_x is the population mean, a_x are the breeding values with variance $\hat{V}_{a_x}$ and e_x represent the residual effects with variance $\hat{V}_{e_x}$.

Following the logic and structure of model 2, in model 4 we fit the phenotypic trait as a fixed effect to estimate and control for its influence on the focal environment variable (i.e., environmental plasticity):

$$x = u_x + \hat{\beta}_{zx}z + a_x + e_x \ (7).$$

Finally, model 5 is the bivariate model that is fitted to estimate the additive genetic covariance $Cov[a_z, a_x]$ between the phenotypic and the environmental variable:

$$[z\ x\ ] = u + a + e \text{ (8).}$$

$$[a_z\ a_x\ ] \sim MVN(0, G)\text{: } \left[\hat{V}_{a_z}\ \widehat{Cov}[a_z, a_x]\ \widehat{Cov}[a_x, a_z]\ \hat{V}_{a_x}\ \right] \text{ (9).}$$

Where $\widehat{Cov}[a_z, a_x]$ represents the estimated additive genetic covariance between the phenotypic and the focal environment, and $\hat{V}_{a_z}$and $\hat{V}_{a_x}$represent the estimated additive genetic variance in the phenotypic trait and focal environment, respectively. *MVN*(0, **G**) represents a multivariate normal distribution where G is the genetic variance-covariance matrix.

For bivariate models, starting values for the variances in the additive genetic (**G**) and the residual (R) covariance matrices were based on the output from the univariate models (Wilson et al., 2010) to improve model convergence. For simplicity, initial values were set to 0.1 when univariate models detected additive genetic variance and set to 0.0000001 when the estimated additive genetic variance was close to 0. Covariance starting values were always 0.0000001.

Results

In general, applying different animal models (i.e., assuming different causal structures) results in the estimation of different parameters (total, direct or indirect additive genetic variance, or a biased estimate that does not correspond with any potential parameter of interest) depending on the specific characteristics of the different biological scenarios. Table S1 in the Supplementary material provides a summary for all possible model-scenario combinations.

Analyzing the standard scenarios with classical animal model structures

When models 1 and 2 were applied to standard scenarios 1-4 where a direct genetic basis for the phenotypic trait and/or phenotypic plasticity was simulated, the expected value for the

estimated genetic variance is equal to the simulated direct additive genetic variance of the phenotypic trait ($E\left[\hat{V}_{a_z}\right] = \sigma^2_{a_z}$. (Supplementary material results, Table S2.) Note that we refer to the expected value of the estimates ($E[\hat{}]$) because each estimate will vary around the value used as input for the simulations because of finite sample size.

What happens if the focal environment has a genetic basis, and it is fitted as a dependent variable in an animal model?

When the presence or absence of a direct genetic basis for the focal environment and/or environmental plasticity were simulated (scenarios 5, 9 and 10) and we applied models with the focal environment as the dependent variable and the phenotypic trait as a covariate (models 3 and 4), the expected value for the estimated genetic variance is equal to the simulated direct additive genetic variance of the focal environment ($E\left[\hat{V}_{a_x}\right] = \sigma^2_{a_x}$; Fig. S2). The estimated strength of environmental plasticity also corresponded to the simulated value ($E\left[\hat{\beta}_{zx}\right] = \beta_{zx}$; Fig. S3, Table S3).

How are genetic variance estimates affected when the focal environment has a genetic basis and the phenotypic trait responds plastically to it?

When we simulated a genetic basis for the phenotypic trait and the focal environment but no plasticity (scenarios 5 and 6) and then applied models with the phenotypic trait as the dependent variable, without or with the focal environment as a covariate (models 1 and 2), the expected value for the estimated genetic variance is equal to the simulated direct genetic variance ($E\left[\hat{V}_{a_z}\right] = \sigma^2_{a_z}$). The estimated phenotypic plasticity also matched the simulated value ($E\left[\hat{\beta}_{xz}\right] = \beta_{xz}$; Figs 2-3; Table S4). Symmetrically, the same happened in scenarios 3 and 6

for the focal environment estimates when we applied models 3 and 4 (Supplementary material results).

In contrast, when we simulated phenotypic plasticity alongside a genetic basis for the focal environment (scenarios 7 and 8) and applied the model with the phenotypic trait as dependent variable and no covariates (model 1), the expected value for the estimated genetic variance is equal to the sum of the simulated direct genetic effects and indirect genetic effects ($E\left[\hat{V}_{a_z}\right] = \sigma^2_{a_z} + \beta^2_{xz}\sigma^2_{a_x}$; Fig. 2; Table S4). On the other hand, when the focal environment was added as a covariate (model 3), the indirect genetic effects of the environment were statistically removed. The expected value for the estimated genetic variance is then equal to the simulated direct genetic variance ($E\left[\hat{V}_{a_z}\right] = \sigma^2_{a_z}$) (Fig. 2, Table S3). For these scenarios, phenotypic plasticity was correctly estimated (Fig. 3, Table S3).

Symmetrically, the same was true for the genetic variance of the focal environment and environmental plasticity estimates in scenarios 11 and 12 when models 3 and 4 were applied (Supplementary material results).

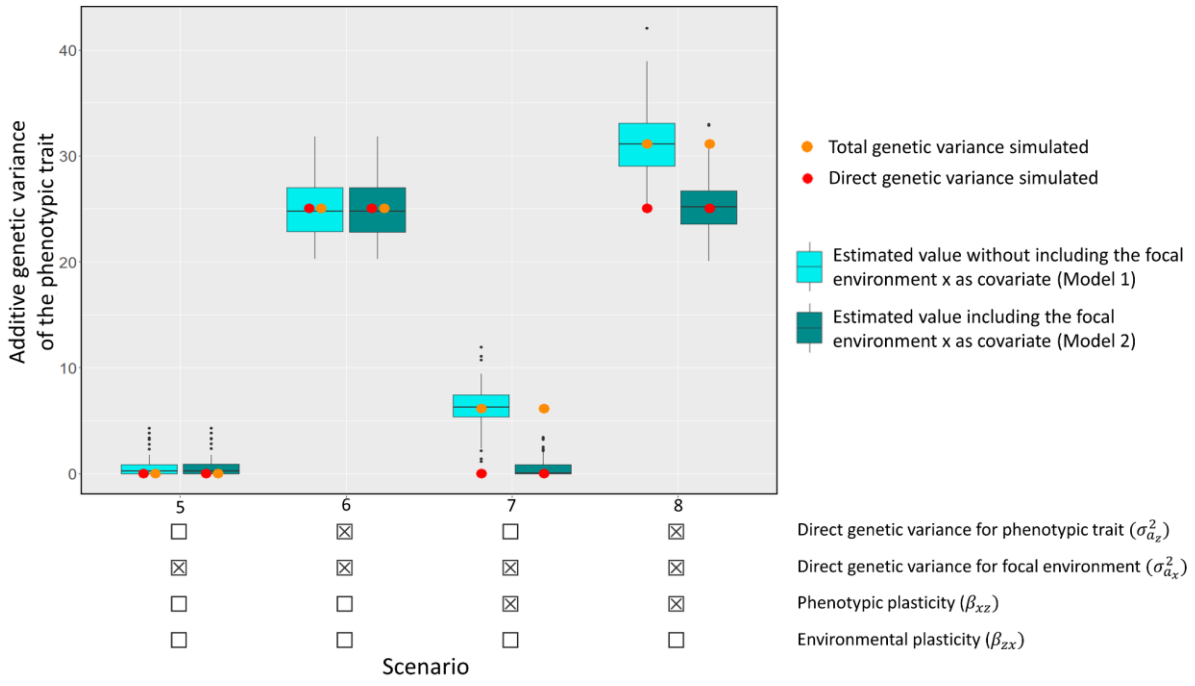

Figure 2. Estimated additive genetic variance of the phenotypic trait for models 1 (phenotypic trait as dependent variable) and 2 (with the focal environment as a covariate) for the simulated values in scenarios 5 to 8. The box plots illustrate the distribution of estimates of the 100 simulations for each scenario (the bottom and the top of the boxes are the first and third quartiles, the middle band is the median, its whiskers extend from the box to highest and lowest points within 1.5 times the interquartile range. Outliers are represented with black dots. Red dots are the simulated direct genetic variances for the focal phenotypic trait. Orange dots are the simulated total genetic variance (direct + indirect; see Discussion). Crossed squares (☒) indicate if non-zero direct genetic variance for the phenotypic trait, direct genetic variance for the focal environment, phenotypic plasticity and/or environmental plasticity were simulated.

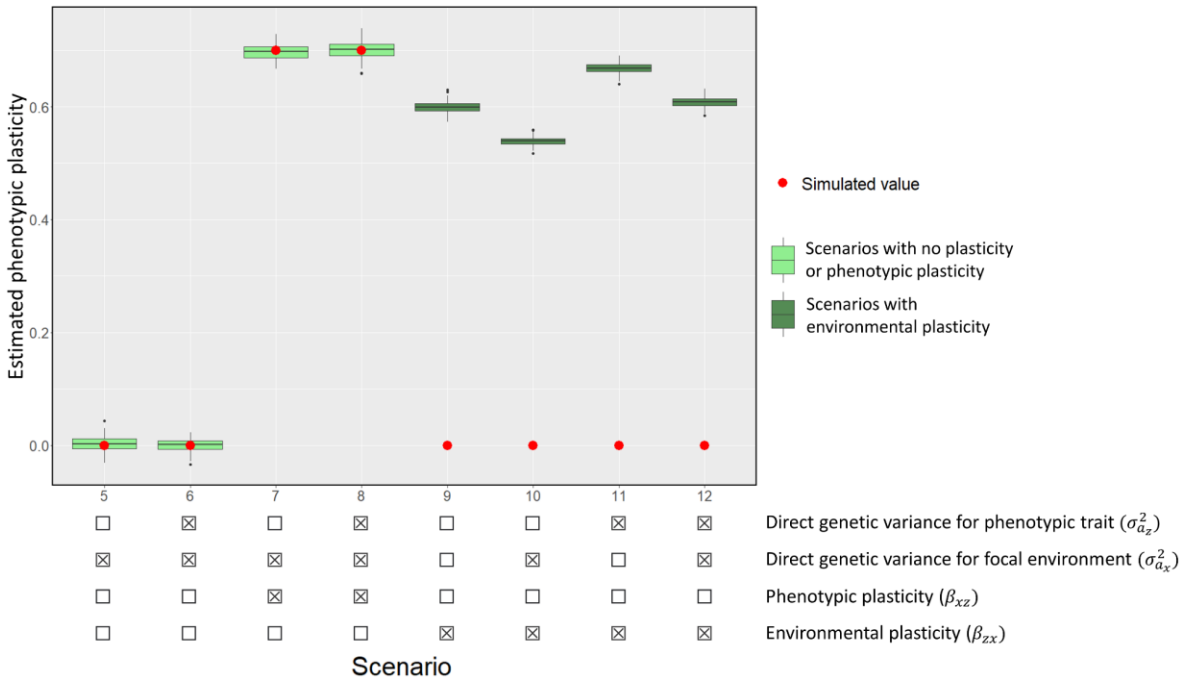

Figure 3. Estimated effects of the focal environment on the phenotypic trait (i.e., strength of phenotypic plasticity), for model 2 (focal environment as covariate). See Fig. 2 for box plot description and legend explanation.

What happens if there is environmental plasticity, yet the classical animal model structures are applied?

When applying the model with the phenotypic trait as the dependent variable and no environmental covariate (model 1) to scenarios where environmental plasticity was simulated (9, 10, 11 and 12), the expected value for the estimated genetic variance is equal to the simulated direct genetic variance ($E\left[\hat{V}_{a_z}\right] = \sigma^2_{a_z}$; Fig. 4, Table S5). However, when we added the focal environment as a covariate (model 2) and thus applied a model assuming the wrong causal relationship (assuming phenotypic plasticity when there is environmental plasticity), phenotypic plasticity was estimated to be present when it was absent (Fig. 3, Table S5).

Moreover, due to this misspecification, the additive genetic variance was misestimated (See Discussion; Fig. 4, scenarios 10-12).

Symmetrically, the same happened for the scenarios 2, 4, 7, and 8 when model 4 was applied (See Supplementary material for results).

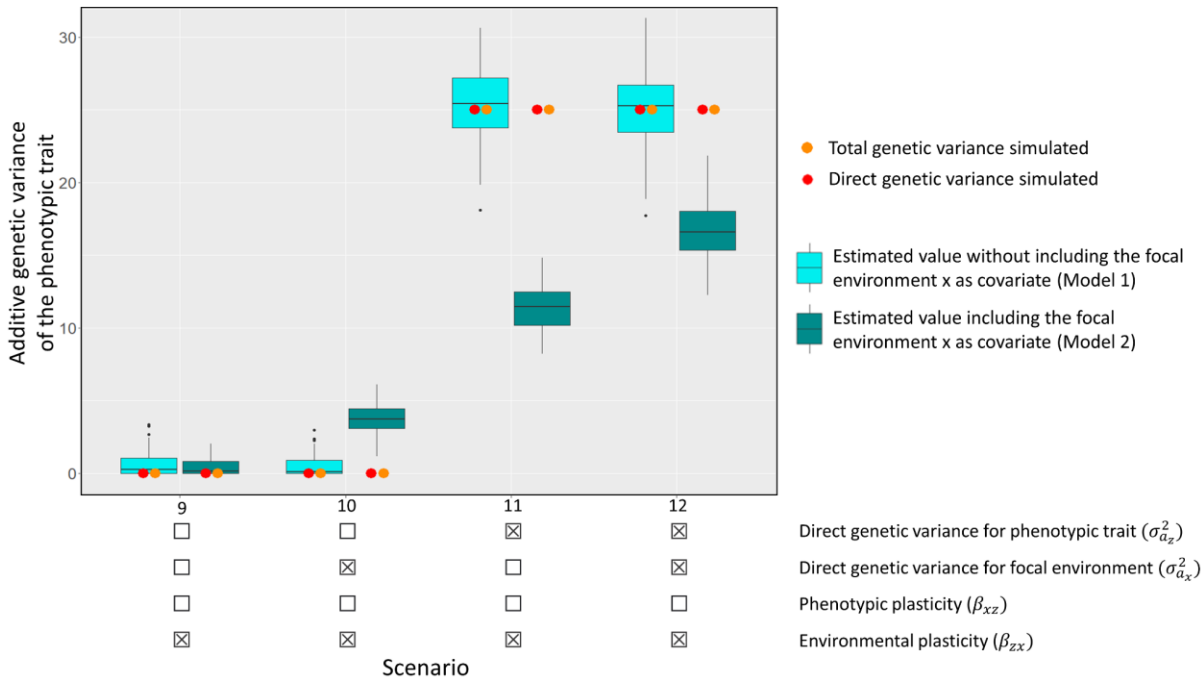

Figure 4. Distribution of the estimated values of additive genetic variance of the phenotypic trait for models 1 (phenotypic trait as dependent variable) and 2 (focal environment as covariate) for the simulated values in scenarios 9 to 12 (see Fig. 2 for box plot description and legend explanation).

What if bivariate models are applied?

When applying the bivariate model (model 5) to all scenarios, estimates for the genetic variances of the phenotypic trait and the focal environment matched estimates by models 1 ($\hat{V}_{a_z}$) and 3 ($\hat{V}_{a_x}$) (i.e., univariate models without a covariate; Supplementary material results).

Moreover, the genetic covariance between the phenotypic trait and the local environment was correctly estimated when it was simulated to exist (phenotypic plasticity together with a genetic basis for the focal environment, scenarios 7 and 8; or environmental plasticity together with a genetic basis for the focal phenotypic trait, scenarios 11 and 12) (Fig. 5).

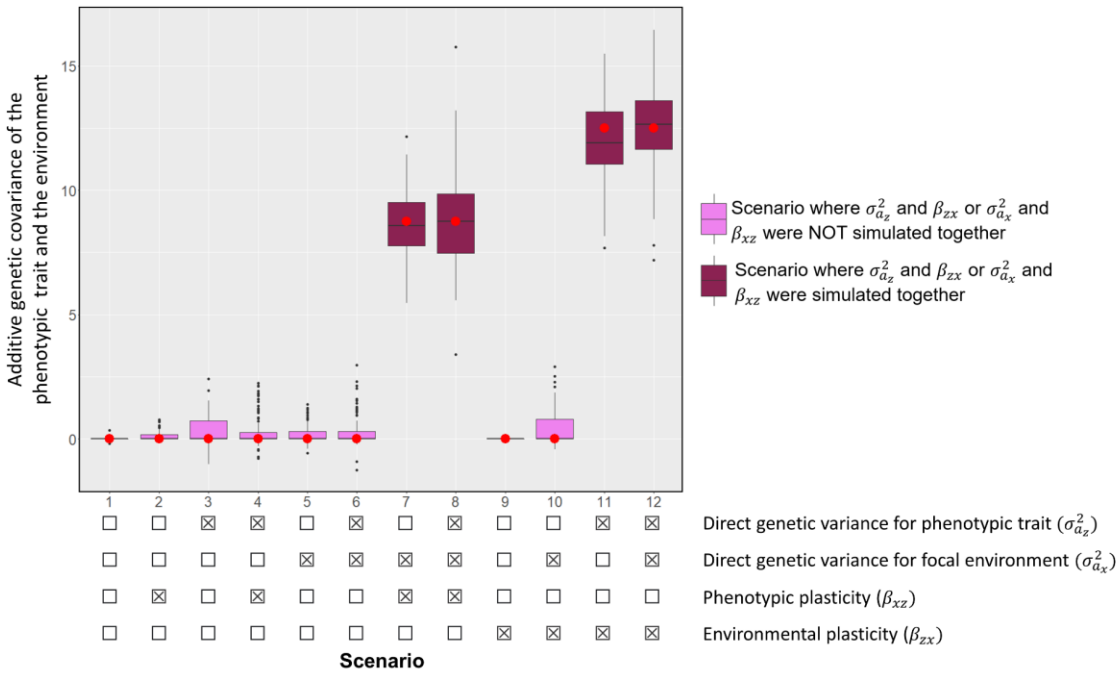

Figure 5. Estimated values of additive genetic covariance between the phenotypic trait and the focal environment (model 5, bivariate model) for the simulated values in all (12) scenarios. Red dots are the simulated additive genetic covariance between the focal phenotypic trait and the focal environment (see Fig. 2 for box plot description and legend explanation).

Discussion

We compared the results of different animal model structures applied to simulated data reflecting different biological scenarios. Our results show: (i) how the genetic basis of the focal environment can be estimated using animal models, (ii) how animal models can estimate not only phenotypic plasticity but also environmental plasticity if the correct model

structure is fitted, (iii) how plasticity can increase the additive genetic variance of the focal trait and how the additive genetic estimates provided by the animal model can be potentially misinterpreted, (iv) how fitting the wrong causal structure can result in wrong inferences about the additive genetic variance and type of plasticity, and (v) how bivariate models can detect a genetic covariance between the phenotype and the focal environment and may help differentiating between alternative scenarios.

The genetic basis of the focal environment can be estimated with animal models

The additive genetic variance of the focal environment can be estimated by fitting it as a dependent variable in an animal model (models 3 and 4). The estimated genetic variance of the focal environment should reflect genetic variation for an individual's preference and ability to choose or adjust its environment (Akcali & Porter, 2017; Edelaar & Bolnick, 2019). This modeling approach allows studying the "heritability" of environmental variables potentially chosen or adjusted by individuals in wild populations (i.e., extended phenotypes) using pedigree or genetic relatedness information. This approach would also be useful whenever the trait underpinning the choice or adjustment of the environment is unknown or cannot be measured directly. As discussed further below, what the estimate provided by the animal model means (direct, indirect or total additive genetic variance of the focal environment) will depend on the model structure.

Social scientists have already recognized that many environments are heritable, since humans select, modify and create environments using behaviors with a genetic basis (Plomin et al., 2016; Saltz, 2019). However, this view has hardly been adopted for wild non-human

populations (see Møller, 2006 and Weber et al., 2013 for exceptions), and animal models have almost never been applied for this purpose (Järvinen et al., 2017; Gervais et al., 2020 and Gervais et al., 2022 are the only exceptions we know of). We hope that researchers will recognize the potential of studying the heritable variation of the environment experienced by individuals by treating it as an extended phenotype. In this way, the focal environment is no longer exclusively modeled as an external ecological context imposing selective pressures, but as one that may be chosen or adjusted by the organism (Edelaar & Bolnick, 2019), and therefore as an integral part of an organism's adaptive potential, with its own genetic basis and evolutionary dynamics. The approach we outline here provides a powerful tool to quantify the heritability of the environment. As more heritability estimates of the environment accumulate, comparative analysis can provide insights on which types of environments have no, low or high heritability, improving our ability to predict evolutionary responses to environmental change.

Animal models can estimate environmental plasticity if the right model structure is fitted

Treating the focal environment as a dependent variable, a phenotypic trait can be fitted as a fixed effect to estimate its impact on the focal environment. This could be expanded to any type of regression that allows estimating the reaction norm of the focal environment, i.e., as a function of the phenotypic trait. The advantage of using animal models in this context is that they take into account the non-independence caused by relatedness among individuals.

In the last decade, research into phenotype-responsive choice of the environment in the form of so-called matching habitat choice (Edelaar et al., 2008) has gained relevance and consolidation (Lowe & Addis, 2019; Camacho et al., 2020), but there are still many open questions. The animal model may be a valuable addition to the toolbox for further study. In

contrast, the effect of variation in individual phenotypes on adjustment of the environment (e.g., niche construction) appears to have been virtually ignored in the scientific literature (Edelaar & Bolnick 2019). The animal model structure we propose might be able to shed some light on this too. As is the case for phenotypic plasticity (Pigliucci, 2005), genetic variation in environmental plasticity is necessary for it to evolve. Thus, estimating genetic variation in environmental plasticity, or in other words G(ene) by P(henotype) interaction is another interesting avenue for future research.

Animal models detect increased genetic variation via pleiotropy / indirect genetic effects

We found that the estimated genetic variance of the phenotypic trait is larger when the phenotypic trait itself is affected by a focal environment that harbors genetic variance (Fig. 2, scenarios 7 and 8). Here, the genes underpinning the chosen or adjusted focal environment have an indirect pleiotropic effect on the plastic phenotypic trait (Fig. 1, b). The estimates of the genetic variance correspond to the sum of the simulated direct genetic effects (the direct genetic basis for the phenotypic trait, $\sigma^2_{a_z}$) and indirect genetic effects (the direct genetic basis for the focal environment, $\sigma^2_{a_x}$, proportional to the square of the strength of phenotypic plasticity, β^2_{xz}). This form of pleiotropy is sometimes called environmental pleiotropy (Paaby & Rockman, 2013; Saltz, 2019). If desired, these indirect effects can be controlled for and filtered out by fitting the focal environment as a fixed effect in the animal model (Fig. 2, scenario 7 and 8, model 2). Doing so allows estimating the direct genetic variance used in our simulations. Note that for real populations, the estimated genetic variance for a trait after controlling for a focal environmental variable would still be the sum of its additive direct genetic variance plus the indirect effects of other unmeasured environmental (and phenotypic) variables affecting the focal phenotypic trait. Therefore, the value and interpretation of the

direct estimated additive direct genetic variance is contingent on the model structure. All of the above applies symmetrically to instances where a focal phenotypic trait with a genetic basis affects the focal environment (scenarios 11 and 12).

A similar issue has been addressed before, when focusing on the covariance between two different phenotypic traits (Villemereuil et al., 2018), showing how the inclusion of a phenotypic trait as a covariate "explains away" some of the additive genetic variance. Generalizing this to a focal environment as a second trait, empiricists should be aware that the environment is not always independent of the organism (i.e., can be an extended phenotype), and that including it in the model changes the interpretation of what is estimated.

Consequences of fitting the wrong causal model

When including a focal environment with a genetic basis as a covariate in the animal model with the intention to disentangle direct and indirect genetic effects, the model structure assumes that the phenotype-environment covariance is due to a causal effect of the environment on the phenotypic trait (i.e., phenotypic plasticity). However, this is problematic when in reality it is the focal phenotypic trait that influences the focal environment (i.e., there is environmental plasticity). In this case, the "classical" structure of the animal model does not reflect the real causal structure of the biological system. When fitting this model, a "false" phenotypic plasticity is estimated (Fig. 3) as a function of:

$$E\left[\hat{\beta}_{xz}\right] = \beta_{zx}\frac{\sigma_z^2}{\sigma_x^2+\beta_{zx}^2\sigma_z^2} = \beta_{zx}\rho \quad (10).$$

Where β_{zx} is the simulated environmental plasticity, σ_z^2 is the variance of the phenotypic trait, σ_x^2 the variance of the focal environment and $\beta_{zx}^2\sigma_z^2$ is the indirect variance caused by

environmental plasticity. The false estimate of the phenotypic plasticity is thus a function of the environmental plasticity β_{zx} and the coefficient ρ, representing the ratio between the phenotypic variance and the total variance in the environment.

Consequently, assuming the wrong causal structure may also result in the additive genetic variance to be misestimated (see Supplementary material for more details). In this scenario, the estimated additive genetic variance of the trait is a function of:

$$E[\hat{V}_{a_z}] \approx \sigma^2_{a_z} + \beta^2_{zx}[\sigma^2_{a_x}\,\rho^2 + \sigma^2_{a_z}(\beta^2_{zx}\rho^2 - \,2\rho)] \quad (11)$$

The second term of the right-hand side of this equation captures the bias caused by fitting the wrong causal structure. The estimated value is dependent on the additive direct genetic variance of both the phenotype and the environment, $\sigma^2_{a_z}$ and $\sigma^2_{a_x}$, the magnitude of environmental plasticity, β_{zx}, and the ratio between the phenotypic variance and the total variance in the environment, ρ (Fig 4, scenario 12). When there is no environmental plasticity (β_{zx} = 0), the estimated additive genetic variance of the phenotype is equal to the additive genetic variance of the focal trait (i.e., $E\left[\hat{V}_{a_z}\right] = \sigma^2_{a_z}$). However, if environmental plasticity is not 0, even if there is no additive genetic variance for the phenotypic trait ($\sigma^2_{a_z}$= 0), a "phantom" additive genetic variance can be estimated when there is additive genetic variance for the focal environment ($\sigma^2_{a_x} \neq$ 0); Fig. 4, scenarios 10 and 11). Furthermore, even in a scenario where there is no additive genetic variance in the environment ($\sigma^2_{a_x}$= 0), if there is indeed some genetic variance in the phenotype ($\sigma^2_{a_z} \neq$ 0), the additive genetic variance in the phenotype will be misestimated as a function of the phenotypic and environmental variance ratio, and the strength of environmental plasticity.

These results highlight the importance of correctly identifying the causal relationship between local environments and phenotypic traits before fitting an environmental covariate in an animal model. Researchers are sometimes tempted to add many environmental variables hypothesized to be affecting the phenotypic trait, because they are believed to cause an overestimation of the genetic variance when related individuals share the same environments (Wilson et al., 2010), or to estimate conditional heritabilities (Villemereuil et al., 2018). However, this could lead to a wrong interpretation of the results if the animal model does not reflect the actual causal structure underpinning phenotypic expression. Previous studies including environmental covariates have almost invariably not taken alternative biological scenarios into consideration. This could have resulted in wrong inferences about the genetic architecture and evolutionary potential of phenotypic traits. Following up the example in the introduction, if boldness is affecting nest site selection (and not the other way around), then treating boldness as a response variable and nest distance to the closest road as a covariate would result in detecting a false influence of the nest distance on boldness (i.e., inferring a false phenotypic plasticity), likely biasing the estimates of genetic variance for boldness. Importantly, these problems are not unique to phenotype-environment relationships, but to any wrongly inferred casual phenotype-phenotype relationship fitted in an animal model.

Role of bivariate animal models

Bivariate animal models allow estimating the total genetic variance of both the phenotypic trait and the focal environment. Moreover, they can estimate the genetic covariance $Cov[a_z, a_x]$ that can arise when the focal environment has a genetic basis and affects the phenotypic trait via phenotypic plasticity, or when the phenotypic trait has a genetic basis and affects the local environment via environmental plasticity (Fig. 5). From the estimates of the

variance-covariance matrix, it is possible to obtain an estimate of the strength of phenotypic and environmental plasticity as:

$$\hat{\beta}_{xz} = \frac{\widehat{Cov}[a_z,a_x]+\widehat{Cov}[e_z,e_x]}{\hat{V}_{a_x}+\hat{V}_{e_x}} \text{ (12).}$$

$$\hat{\beta}_{zx} = \frac{\widehat{Cov}[a_z,a_x]+\widehat{Cov}[e_z,e_x]}{\hat{V}_{a_z}+\hat{V}_{e_z}} \text{ (13).}$$

However, it would be necessary to know which type of plasticity (genetic or environmental) is acting in the studied population. Furthermore, the sign of the genetic and residual covariance should be the same if there is only one process underpinning the relationships between trait and environment.

It is also possible to calculate the direct genetic variance of the phenotypic trait after controlling for the indirect effects of the environmental variable and vice versa, for instance, in our simulation:

$$\sigma^2_{a_z} = E\left[\hat{V}_{a_z} - \frac{\widehat{Cov}[a_z,a_x]}{\hat{V}_{a_x}}\right] = E\left[\hat{V}_{a_z} - \hat{\beta}^2_{xz}\hat{V}_{a_x}\right] \text{ (14).}$$

$$\sigma^2_{a_x} = E\left[\hat{V}_{a_x} - \frac{\widehat{Cov}[a_z,a_x]}{\hat{V}_{a_z}}\right] = E\left[\hat{V}_{a_x} - \hat{\beta}^2_{zx}\hat{V}_{a_z}\right] \text{ (15).}$$

However, these calculations will be correct only if we know whether environmental or phenotypic plasticity is causing the genetic covariance between the phenotypic trait and focal environment, and thus depend on knowing the correct causal structure.

Thus, bivariate animal models with the focal phenotypic trait and the focal environment as response variables could be applied as a first step to disentangle alternative biological scenarios. First, it could indicate to what extent the phenotypic trait and the focal environment have a genetic variance. Second, if a genetic covariance is detected, there could be some type of plasticity influencing the total genetic variance of one of those traits. Biological insight or additional (experimental) data sets might then help to clarify the causal relationships, i.e., whether there is phenotypic plasticity, environmental plasticity or other types of non-random assortment of genotypes in their environment (see below).

Additional sources of genotype-environment covariance

Genotype-environment covariance may arise due to biological processes other than plasticity. One is divergent natural selection. When phenotypic traits (genotypes) are divergently selected across local environments, a gene-environment correlation is generated and a genetic covariance for the phenotypic and environmental traits is detected by the animal model. Relatives are more similar phenotypically and therefore more likely to occur (survive and reproduce) in the same habitats. Thus, a genetic variance for the environment could also be detected. Therefore, the animal model could detect non-existing phenotypic or environmental plasticity, whereas in reality the covariate does not influence the *development* of the focal trait, but its continued *presence* (via differential survival or reproduction).

Other scenarios where a genetic covariance could emerge are those where plastic habitat choice is present. That occurs when a preference for an environment is induced by an environmental cue during ontogeny, i.e., imprinting or learned habitat choice (Akcali & Porter, 2017; sometimes also called social learning, e.g., Lillie et al., 2018). For example, in some species,

individuals choose environments similar to those they experienced during their natal stage (the period between birth and independence from the parent). Therefore, they choose an environment similar to the one their parents chose (e.g., Nielsen et al., 2013). This causes parents and offspring to share local environments, which again could generate a gene-environment correlation. Something similar happens when there is a degree of philopatry, i.e., dispersal does not randomize relatives across environments (Ducros et al., 2020; Gervais et al., 2022).

Finally, genetic covariance between the phenotypic trait and the environment will also emerge when there are loci that directly affect both traits (i.e., there is direct pleiotropy).

Other limitations

We acknowledge here that we only addressed simplified scenarios compared to those in real biological systems. First, the phenotypic trait and the focal environment are assumed to follow a Gaussian distribution and plasticity is linear. The effect of non-Gaussian traits and/or non-linear reaction norms on the estimation and potential misinterpretation of genetic parameters can be more complex and may need a specific treatment (see Morrissey (2015) and Villemereuil et al. (2018)). Second, for all models we assumed that the expression of the phenotypic trait and the focal environment (extended phenotype) are independent of the frequency and density of other phenotypes, which are roughly constant across our simulations anyway. Finally, we just presented bivariate scenarios where only two traits are involved. Increasing the number of traits and environments would greatly increase the number of potential causal structures. Moreover, these causal structures can even change across different

environments (e.g., Tonsor & Scheiner 2007). Nevertheless, this paper provides some general conclusions that can be the starting point for further studies.

Advice to empiricists: estimating total, direct, indirect or uninterpretable additive genetic variance

Researchers should decide what kind of additive genetic variance they want to estimate (total, direct and/or indirect) before performing any kind of analysis, to avoid misinterpretation of the heritability of the focal trait. First, the total additive genetic variance for the focal trait can be estimated by not including any covariate with a genetic basis in the model or by performing a bivariate animal model. Second, the direct additive genetic variance of a focal trait, i.e., what remains after removing the indirect effects of a covarying trait (an extended phenotype, or any other correlated phenotypic trait for that matter), can be estimated by fitting the covariate as a fixed effect in the animal model or by performing a bivariate animal model (Equations 14 and 15). In the same way, by subtracting the estimate of direct additive genetic variance from the estimated total additive genetic variance, the indirect genetic effects of the covariate can be estimated.

However, relevant caution is needed in two additional steps prior to analysis. First, when fitting a focal environment as a fixed effect in the animal model. Both total or direct (total minus indirect genetic effects) genetic variance could be estimated depending on whether the environment has a genetic basis or not (e.g., when applying model 2 for scenarios 4 and 7 respectively). Thus, it is necessary to know if the focal environment is heritable. Not being aware of this could lead to part of the genetic variance being wrongly interpreted to reflect non-heritable environmental effects (Gervais et al., 2022). Second, when choosing which causal

structure is fitted when making inferences about the process underpinning the covariance between phenotypes and environments (or two phenotypic traits). Fitting the wrong structure results in a misinterpretation or even miscalculation of the genetic parameters and which type of plasticity is acting (e.g., when applying model 2 to scenarios 10-12). Biological insight or additional measures and experiments might be needed. If a phenotype-environment correlation is detected in a study population, we should first rule out the possibility that the environmental variable may be (partially) genetically inherited (or non-genetically inherited for that matter). If that is not possible, we should proceed to test experimentally the existence of phenotypic or environmental plasticity by manipulating the environment or the phenotypic trait (see Camacho et al., 2020 for an example of testing for environmental plasticity). If we are uncertain about the correct causal structure, we should proceed with great caution when making inferences about the obtained estimates of genetic variance when fitting a heritable variable as fixed effect, and at the very least the assumptions and possible consequences underlying a chosen model structure should be discussed. If we ignore the potential mechanisms underpinning gene-environment correlations, we could arrive at misleading conclusions about the adaptive potential and expected evolutionary dynamics of the phenotypes of our study organisms and, therefore, on their ability to cope with environmental change.

Chapter II: Quantifying heritability of the environment using animal models: a case study

This chapter reproduces entirely the manuscript:

Munar-Delgado G., Nicolaus, M., Both, C. & Edelaar, P. Quantifying heritability of the environment using animal models: a case study. *In preparation*

Abstract

Evolutionary ecology has traditionally studied how natural selection shapes the phenotypes of individuals in response to their environment, which increases population fitness. It is also well known how habitat choice can affect individual local adaptation. However, recent work has highlighted the incompleteness of the link between habitat choice and its evolutionary consequences. By treating the selected habitat as an extended phenotype, the evolutionary focus can be shifted to how the extended phenotype evolves to match the organisms' phenotypic traits. Theoretical approximations suggest that animal models could be used to estimate the heritability of the extended phenotype, providing insights into its evolutionary dynamics. Here we use data from a long-term study of a pied flycatcher population (*Ficedula hypoleuca*) to test the use of animal models for the estimation of the heritability of an extended phenotype i: the vegetation around the nest box. We also applied animal models to different subsets of the population (based on philopatric status) to test which mechanism might be causing the inheritance of the extended phenotype, if any. We also ran simulations to randomize the nest box and its surrounding vegetation for the different population subsets, thereby eliminating the effect of habitat choice, and used the animal models to identify the sources of variation of the extended phenotype.We confirmed that animal models can be used to estimate the local habitat heritability in natural populations and found a significant habitat heritability for our pied flycatcher population. Moreover, subset analysis and subset randomization both indicated that the observed heritability was caused by philopatry. Thus, we propose that animal models can be used to estimate the heritability of the extended phenotype and also to disentangle the mechanism(s) causing its inheritance. By acknowledging that organisms can influence the habitat they experience to increase their adaptation and by focusing on the evolution of

extended phenotype, we should be able to better understand how population adaptation increases and how it evolves.

Key words: additive genetic variance, animal model, extended phenotype, habitat inheritance, habitat choice, philopatry

Introduction

Traditionally, adaptation has been seen as the process of natural selection shaping populations to match pre-existing environmental templates (Darwin, 1859; Schluter, 2001). Consequently, the causal relationship of adaptation has been considered as unidirectional: the environment affects the organisms, but not the other way around. However, recent studies have synthesized and highlighted how organisms can affect their local environment by either adjusting or selecting it to match their 'phenotypic templates' (Edelaar et al., 2023; Edelaar & Bolnick, 2019; Laland et al., 2016). When individuals within a population differ in the environments that best match their own characteristics (Bolnick et al., 2002), their local performance will vary across distinct environments. Under such circumstances, natural selection should favor mechanisms that facilitate individuals to select environments that best match their phenotypes (Davis & Stamps, 2004), or to modify them (Callahan et al., 2014). Thus, the causal relationship between organisms and their environment in adaptation terms can be bidirectional, the organism and the environment can each be changed to match the other (Edelaar et al., 2023; Laland & Sterelny, 2006).

To highlight the capacity of organisms to adjust their environment, the adjusted environment has been called an 'extended phenotype' (Dawkins, 1982). The extended phenotype has traditionally referred to the expression of the genotype in the external environment, any change made in the environment by the organism that affects its own fitness. An example of this is the nest of birds. Birds adjust their local environment by constructing a nest which, in fact, affects their reproductive success. Thus, natural selection should favor the most successful nests and if the bird building capacity is heritable, the extended phenotype (the nests) is expected to evolve. On the other hand, extending this logic, the habitat selected by the organism has also

been recently approached as an extended phenotype since it (partially) depends on the organism (Edelaar & Bolnick, 2019; Munar-Delgado et al., 2023) and potentially affects fitness. Following the nest example, when a bird selects a nest site (such as a nest cavity), and its characteristics affect reproductive success, the outcome is similar to that of nest building, and nest site selection is expected to evolve. Here we focus on the selected habitat as an extended phenotype which can be inherited and therefore have its own evolutionary dynamics (Edelaar et al., 2023).

Evolutionary quantitative genetics has traditionally focused on the adaptive evolution of phenotypic traits, estimating their heritability and evolutionary potential. However, recently, Munar-Delgado et al. (2023) have suggested to approach the habitat as a potential extended phenotype and to apply the quantitative genetics perspective using animal models. Animal models are mixed models widely used in quantitative genetics to estimate the heritability of phenotypic traits (Kruuk, 2004; Lynch & Walsh, 1998). In conventional application of these models, the environment may be fitted as a covariate to control for its effects on the phenotypic trait (i.e., to model phenotypic plasticity). However, when we consider that the environment is an extended phenotype and it is instead fitted as a dependent variable in the animal model, its heritability can also be estimated (Gervais et al., 2020, 2022; Järvinen et al., 2017; Munar-Delgado et al., 2023). Additionally, Munar-Delgado et al. (2023) showed how the heritability of phenotypic traits increases when they are affected by a heritable extended phenotype via phenotypic plasticity. So even if the focal trait is a phenotypic trait and the habitat inheritance is not of primary interest, not taking into consideration that the habitat is heritable could bias the interpretation of estimates of phenotypic trait heritabilities. Thus, applying this quantitative genetic methodology to environmental traits seen as influenced by the organism should help

to: i) understand if and how environments are heritable and ii) disentangle the evolutionary dynamics between the chosen habitat and other phenotypic traits.

If the chosen habitat is heritable, it can be inherited via different habitat choice mechanisms: direct genetic habitat choice, plastic habitat choice and matching habitat choice (Akcali & Porter, 2017; Ravigne et al., 2004). Direct genetic habitat choice refers to habitat choice directly mediated by preference alleles. This process is able to drive adaptation when preference alleles differ among individuals and are coupled (though physical linkage or linkage disequilibrium) with other phenotypic differences that increase the ecological match with the preferred habitat. In this case, the chosen habitat can be seen as genetically determined and to evolve via natural selection acting on variation in habitat use that affects individual fitness (Jaenike & Holt, 1991). Plastic habitat choice refers to habitat choice driven by individual preference induced during its ontogeny by environmental cues (as habitat imprinting). This kind of habitat choice is adaptive when offspring resemble their parents phenotypically; in that case preferring the habitat that their parents used with success is likely successful for the offspring as well. Finally, matching habitat choice is a performance-dependent type of habitat choice where individuals assess their local performance in different habitats and based on that settle where it is highest (Edelaar et al., 2008), thereby increasing individual phenotype-environment match and local adaptation. Thus, besides estimating the heritability of the environment, identifying the inheritance mechanism would provide insight in how it can evolve.

In this study, we applied animal models to estimate the heritability of the environment using an extensive dataset from a long-term study of a pied flycatcher (*Ficedula hypoleuca*)

population. The dataset contains pedigree information and information about each individual's local habitat, which was defined as the composition of the tree vegetation around the nest box used as a breeding adult. By treating the vegetation composition as an individual's extended phenotype, we can estimate its heritability. We expect to be able to apply animal models to estimate the heritability of breeding site vegetation composition.

We also investigated the mechanism involved in any habitat heritability. So far, there is no evidence for direct genetic habitat choice (variation in genetic preference for a specific vegetation composition) for this species. In contrast, it is well known that early experiences in the natal site have effects on habitat choice for pied flycatchers, causing individuals to select breeding sites similar to their natal sites. This seems to support that flycatchers have plastic habitat choice (i.e., based on imprinting), which leads to philopatry (Camacho et al., 2016; Chernetsov et al., 2006). Philopatry in itself could also be a mechanism causing parent-offspring similarity in habitat, when nearby habitat patches are more similar than distant habitat patches (i.e., when there is positive spatial autocorrelation). Hence, when dispersal distances are not long enough to break the pattern of spatial autocorrelation, then this will cause the environment to be heritable. To disentangle the effects of habitat choice versus limited dispersal as the cause for heritability of the environment, we separately estimated heritability for philopatric and non-philopatric individuals (i.e., dispersing within or between study plots). Additionally, to ensure that applying animal models to these relatively small population samples did not reduce statistical power and that interpretations were not biased, we ran simulations of different scenarios in which the nest box locations of different population subsamples (based on philopatry status) were randomized. By doing this, we were able to

remove the effect of habitat selection in the different groups and test where any observed heritability at the level of the total population came from.

Methods

Study species

The pied flycatcher is an insectivorous passerine that migrates long distances, wintering in Sub-Saharan Western Africa and breeding in temperate and boreal forests across Europe. The species easily adopts nest boxes for breeding and is present in habitats ranging from deciduous to coniferous forests.

Study sites

Our study was carried out in Drenthe in the Netherlands (52º49'N, 6º35'N). The study population was established in 2007, comprising twelve distinct study plots. Each of these study plots was equipped with 50 or 100 nest boxes, totaling 1,050 boxes. The distance between adjacent plots varied between 2.6 to 7 km, with the most distant plots being 18 km apart in the north-south direction and 12 km apart in the east-west direction.

Data collection

Arrival Date. Since 2007, the arrival of individuals has been monitored at least once every other day starting from the beginning of April. This process has been described in detail in Both et al. (2016). During each observation session, the focal study plot was traversed on foot, and all evidence of pied flycatcher presence was recorded. The observations were carried out from shortly after sunrise until around noon. The arrival date of male individuals was

determined as the first day of observation when the study plot was visited daily. The arrival of females was recorded as the date when they formed a pair since previous studies have demonstrated that females typically form pairs within a few hours of arrival (Dale et al. 1992).

Pedigree. On day 7 after nestlings had hatched (hatch date = 0), parents were caught using a spring trap and identified or ringed (if unringed) in each box. On the same day, nestlings were ringed. Thus, virtually all breeding birds and nestlings are formally identified with their individual ring number, which allows for the construction of a robust population pedigree. For unringed birds that were caught for the first time, the minimum age was estimated, making a distinction between first-year birds and older ones (Jenni and Winkler, 1994).

Breeding individual status. Individuals were initially classified as either "recruits," indicating they were born in our study area (in any of the 12 plots) and later found breeding within this area, or as "immigrants," indicating they were breeding in the study area but were not locally ringed (either originally unringed or ringed outside the study plots at >20 km). Then, for each year of observation, recruits were further classified as either "philopatric," indicating they were breeding in the same plot where they were born, or as "disperser," indicating they were breeding in a non-natal study plot. It is worth noting that the same individual could hence be classified as philopatric and disperser in different years.

Breeding site vegetation composition. In a subset of 7 plots, the vegetation composition was measured around all the boxes present in the study sites. The average distance between nest boxes and foraging sites in the study plots was 13 m (Oosting, unpublished data), (although

longer flights are difficult to track so this average distance is probably somewhat underestimated). For this reason, the vegetation composition was scored in a 15 m radius around each focal nest box tree. All tree species were counted, and the proportion of deciduous trees was calculated. The vegetation composition was collected by 7 observers, and a subset of 10 boxes was scored by all observers to check for observer effects. The data showed high interobserver correlation coefficients, implying that the measurements are not biased (Nicolaus et al., 2019). Vegetation composition around the nest box was the focal local environment that was treated as a dependent variable (i.e., as an extended phenotype).

Statistical analysis

Study plots variance. We estimated the variance of breeding site vegetation composition for each plot and between plots. We did this to predict if philopatry could create enhanced parent-offspring similarity in breeding site vegetation composition.

Quantitative Genetics Analyses and Animal models. All mixed-effect model analyses were performed using ASReml-R version 4.1.0.130 (Butler, 2020) using a restricted maximum-likelihood approach. The social pedigree was constructed using all available information on individuals that were marked in the nest boxes between 2007 and 2022. The pedigree was built based on the assumptions that all immigrant birds are unrelated to any other individual, and that the observed parents in the nest are also the genetic parents. Since extrapair paternity is not common in this species (<5%, Brommer et al., 2010), this last assumption should have little effect on heritability estimates (Charmantier & Réale, 2005). Maximum pedigree depth was nine generations. For univariate models, we fitted year, individual identity and individual

additive genetic merit (i.e., breeding value) as random effects to decompose the extended phenotypic variance (V_{EP}) into among-year variance (V_Y), additive genetic variance (V_A) and permanent environmental variance (V_{PE}), and residual variance (V_R). Thus, the variance of the extended phenotype was estimated as $V_{EP} = V_A + V_{PE} + V_Y + V_R$, and then we calculated the relative importance of each variance as (i) narrow sense heritability of the extended phenotype $h^2 = V_A/V_{EP}$, (ii) permanent environmental variance $pe^2 = V_{PE}/V_{EP}$, , (iii) year variance $y^2 = V_Y/V_{EP,}$ and (iv) residual variance $r^2 = V_R/V_{EP}$.

When applying the animal model to the different data subsets, if all the heritability for the total population is due to philopatric individuals (those that stay in the natal study patch), we should only observe heritability when applying the animal models to that subset. On the other hand, if there is some degree of other habitat choice, we should also observe heritability when applying the models to the non-philopatric individuals.

Finally, we ran a binomial animal model for philopatry (philopatric vs non-philopatric status). We classified both dispersers and immigrant individuals as non-philopatric. For this model we fitted year, individual identity and individual additive genetic merit (i.e., breeding value) as random effects to decompose the phenotypic variance (V_P) into among-year variance (V_Y), additive genetic variance (V_A) and permanent environmental variance (V_{PE}), and residual variance (V_R). V_R from the binomial model was fixed at $\pi^2/3 = 3.29$ (Falconer and Mackay, 1996). Thus, the variance of the phenotype was estimated as $V_P = V_A + V_{PE} + V_Y + 3.29$, and then we calculated the narrow sense heritability of the phenotype $h^2 = V_A/V_P$. We also estimated philopatry heritability for males and females separately.

Simulations

Simulations were based on randomization of individual breeding nest boxes together with the local environment associated with them (i.e., the vegetation composition). Randomization in principle removes the effect of habitat choice. In different simulations, we randomized nest boxes for different population subsets and combinations of them (philopatric, n = 621; disperser, n = 522; and immigrant individuals n = 2822) while maintaining the original data for the rest of the population. We varied the pool of potential nest boxes that were assigned to each individual (Supplementary material Methods). For half the simulations, breeding individuals were assigned a random nest box out of the ones that were occupied by any pied flycatcher (including itself) that specific year, but only out of the set of boxes in the same plot (within-plot randomization). This removes the effect of habitat choice. For the other half of the simulations, breeding individuals could get a nest box occupied in any of the 12 study plots that year. This removes the effect of philopatry, and habitat choice. By only including nest boxes that were occupied by pied flycatchers each specific year, we ensured that those nest boxes were truly acceptable for individuals to breed that year. Additionally, the pool of potential nest boxes for each individual was dependent on their arrival date (if their arrival date was unknown, individuals were assigned a random arrival date from the pool of arrival dates in that specific year). This is because if certain territories are preferred, these may become scarcer as more individuals have chosen a box. Therefore, individuals could only get a nest box that was unoccupied at their arrival date. In this way, individuals that arrived on day 1 of a specific year could get any of the nest boxes occupied that year, while those arriving on the last day could only get any of the nest boxes that were being occupied that same day. Finally, to increase the randomness of the assignments and because we do not investigate competitive interactions other than depletion by arrival date, nest boxes were assigned without replacement; the same nest box could be assigned several times to different individuals. Overall, following

these criteria, we conducted eight different simulation designs (i.e., eight different scenarios) by changing which population subset was being randomized (philopatric, disperser or immigrant individuals) and whether the randomized nest boxes for each individual were restricted to the individual's breeding plot or not. Thus, we were able to remove habitat choice and philopatry effects for each group separately.

We simulated each different scenario 1000 times, and each resulting new dataset was analyzed using animal models to decompose the extended phenotypic variance (see below). For each scenario, we first calculated the h^2, pe^2, y^2 and r^2 ratios for each simulation and then extracted their mean from the 1000 simulations.

In these simulations, if heritability is only due to philopatry, heritability should only decrease when randomizing the nest box location of philopatric individuals between patches. If it decreases when only non-philopatric individuals are randomized, it would mean that another type of habitat choice is also acting.

Results

Study plot variance

We found that the variance of breeding site vegetation composition between plots was higher than the variance within plots (Table 1).

	Plot							
	2	5	6	7	8	10	12	Between
Variance	254.17	770.51	680.57	529.59	403.78	629.26	1.02	781.31

Table 1. Breeding site vegetation composition variance within each plot and between plots.

Observed heritability of breeding site vegetation composition in relation to philopatry

We found a highly significant heritability for breeding site vegetation composition (0.19 ± 0.04) (estimate ± SE). The heritability was similar for males and females (males: 0.34 ± 0.09; females: 0.23 ± 0.09) (Table S1).

Heritability depended on the dispersal status of individuals. For non-philopatric individuals (immigrants + dispersers), there was no heritability for breeding site vegetation composition (0.00 ± 0.00), and all individual repeatability was estimated to be due to permanent environment effects (0.44 ± 0.03) (Table S1)). In contrast, for philopatric individuals we found a high heritability (0.38 ± 0.10) and no permanent environment effects (0.00 ± 0.10) (This last model output did not fully converge properly, so the estimate is less reliable). The heritability was similar for male and female philopatric individuals (males: 0.40 ± 0.07; females: 0.39 ± 0.09; Table S1).

Heritability estimates in different randomization scenarios

We found negligible change in the estimated heritability of breeding site vegetation composition in simulated scenarios where individuals were randomly assigned a nest box from their natal breeding plot (Fig. 1a; Table S2). These results suggest that, for this population of pied flycatchers, breeding in a randomly assigned box from their natal breeding plot would not affect the inheritance of vegetation composition.

In contrast, we did observe large changes in heritability estimates in simulated scenarios where individuals were assigned a random nest box from any study plot (Fig. 1b; Table S2). Notably, we observed an almost complete elimination of heritability when the breeding nest boxes of philopatric individuals were randomized, indicating that philopatric individuals breeding in their natal plot are responsible for the observed vegetation composition heritability. We also found a considerable decrease in heritability when immigrant individuals (representing the majority of the population) were included in the randomizations. This is because when philopatric individuals have immigrant parents (or ancestors) whose breeding box is randomized, the parent-offspring similarity for the extended phenotype is also reduced (we don't randomize offspring but we do randomize parents) and heritability decreases. Finally, as expected, when all individuals were assigned a random nest box, almost all the observed variance of the extended phenotype was attributed to residual effects.

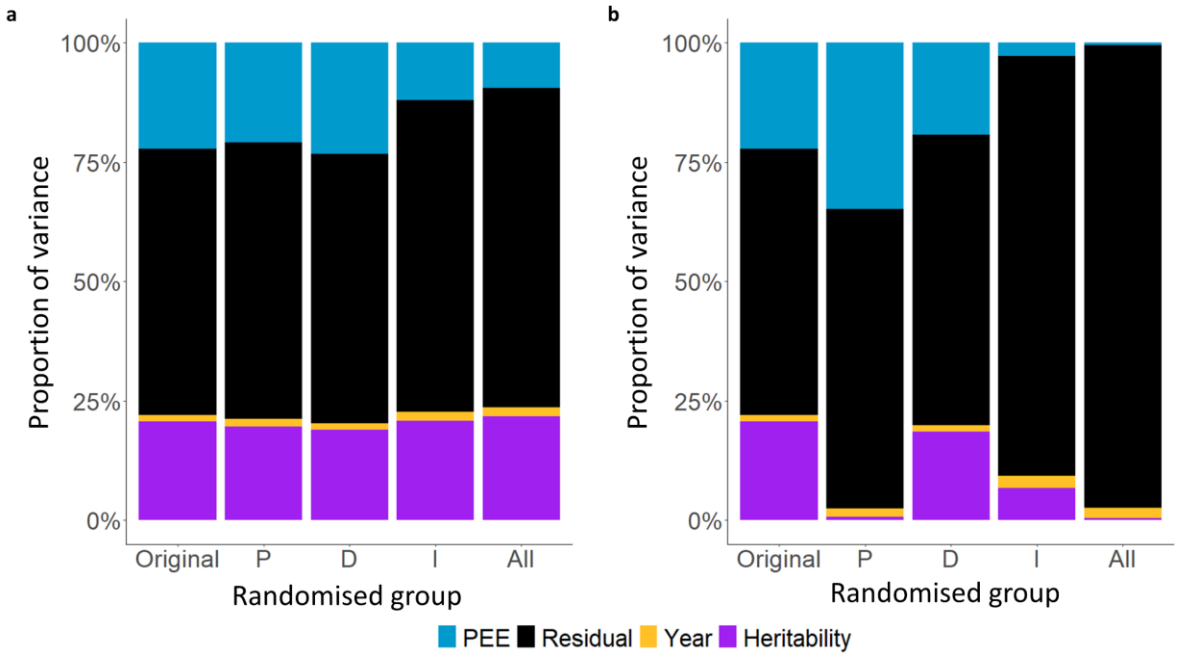

Figure 1. Breeding site vegetation composition (i.e., extended phenotype) variance partitioned into heritability, permanent environmental effects (PEE), among-year variance (Year) and residual variance for the different datasets: original dataset without randomization (Original),

or randomizing philopatric (P), disperser (D), immigrant (I), or all individuals. For the simulated scenarios, plotted values are the means from the 1000 independent simulations. In **a**) randomized breeding individuals were assigned a random nest box from their original breeding plot while in **b**) they were assigned a random nest box from any of the study plots.

Philopatry heritability

Regarding philopatry itself (breeding in the natal plot or not), we found a significant heritability for natal philopatry (0.27 ± 0.04), with no significant differences in heritability between males and females (males: 0.27 ± 0.08; females: 0.38 ± 0.09).

Discussion

Heritability of the environment as an extended phenotype

This study aimed at evaluating the use of animal models to estimate the heritability in a wild population of an aspect of the environment, as an extended phenotype. As expected (Munar-Delgado et al., 2023), we were able to fit animal models with the focal local environment as a dependent variable and estimate its heritability. We found a heritability of the breeding site vegetation composition of 0.19 ± 0.04. This is lower than the mean heritability estimated for morphological, behavioral, physiological, and life-history traits using animal models (0.31 ± 0.03; Postma, 2014) but still statistically different from 0. The first direct consequence is that breeding site vegetation composition can evolve, if it affects fitness (i.e., if it affects the phenotype-environment match). The influence of breeding site vegetation composition on pied

flycatchers' fitness is well established, as they rely on insect peaks in their breeding area to feed their offspring (Burger et al., 2012; Samplonius et al., 2016; Sanz et al., 2003), and different vegetation types (deciduous trees vs non-deciduous) can differ strongly in food availability throughout the breeding season (Both et al., 2009). The temperature increase due to climate change has caused an advance in insect peaks faster than pied flycatchers are able to adjust their laying date via phenotypic plasticity (Both, 2008; Both et al., 2006; Both & Visser, 2001). However, individuals breeding in sites with less deciduous vegetation have a minor need to adjust the timing of breeding because of the relative lack of seasonal insect peaks (Burger et al., 2012). Thus, inheriting breeding site vegetation composition can have direct effects on individuals' fitness in a changing world. Although it is thought that pied flycatchers usually prefer to breed in deciduous patches (Lundberg & Atalo, 1992; Siikamäki, 1995), there might be some fitness trade-offs that maintain this environmental variability and heritability. For example, it could be possible that in warmer years, when pied flycatchers are unable to accordingly adjust their laying date to match the caterpillar peak in deciduous habitats (Both et al., 2006), flycatchers inheriting non-deciduous habitats have a higher reproductive success and, thus, non-deciduous habitat would be positively selected (Both et al., 2006). Thus, an additional step in disentangling this could be estimating the selection differential for the habitat.

Pied flycatchers breeding in deciduous habitats mostly rely on caterpillars (Sanz, 1998) while individuals in more coniferous habitats rely more on flying insects (Lundberg et al., 1981). This difference in diet provisioning during growth as nestlings could affect individuals' phenotypic traits via phenotypic plasticity (Buchanan et al., 2022; Lindström, 1999; Monaghan, 2007), which can also affect their fitness. A direct consequence of the effects of the heritable extended phenotype on other phenotypic traits of the individuals via phenotypic plasticity is

that it also increases the heritability of those phenotypic traits (Munar-Delgado et al., 2023; Saltz, 2019). For example, the heritability underpinning the expression (selection) of the breeding site vegetation composition could indirectly affect the heritability of fledgling body mass (that are fed with different insects while in the nest). In fact, this could occur with any of the phenotypic traits affected by breeding site vegetation composition. Researchers should be aware of this when trying to disentangle genetic and environmental effects shaping the evolution of pied flycatcher phenotypic traits. In quantitative genetics, when estimating the heritability of a phenotypic trait, is a common practice to add to the animal model all the environmental variables hypothesized to affect the phenotypic trait, because it is thought to avoid overestimation of the heritability (Wilson et al., 2010). However, when the environmental variable included as a covariate is heritable, its effects on the phenotypic trait heritability would be statistically removed and the estimated heritability would decrease (Munar-Delgado et al., 2023). Thus, it is necessary to take this into account when interpreting the estimates provided by animal models.

As a summary, all these environment-phenotype interactions suggest that breeding site vegetation composition influences individual fitness and thus it should evolve to improve the environment-phenotype match. In other words, that habitat choice could lead to adaptive evolution of habitat use (Edelaar et al., 2023).

Disentangling the mechanism behind the inheritance of the habitat

We also aimed at identifying the mechanism driving habitat heritability in our pied flycatcher study population. Applying animal models to different subsets of the population based on their dispersal status, we observed that the heritability as estimated for the whole population's

extended phenotype could be attributed solely to philopatric individuals. Testing for heritability in non-philopatric individuals, including dispersers and immigrants, resulted in a heritability estimate of zero (0.00 ± 0.00). However, when we applied the animal model only to the subset of philopatric individuals, heritability was estimated to be higher than for the total population. This indicates that the heritability of breeding site vegetation composition was due to philopatric individuals, which represented only 16% of the observations in our dataset.

The impact of philopatric individuals on the observed heritability was supported by the simulation results. When dispersing individuals were included in the randomizations (representing 13% of the observations) and thereby any habitat choice by them was removed, the mean heritability estimate was similar to the heritability estimate from the original dataset. This suggests that dispersing individuals were not exerting any habitat choice that was promoting habitat inheritance. When philopatric individuals were assigned a random nest box from their original breeding plot, we did not observe any change in the extended phenotype heritability. However, when those individuals were assigned a random nest box from any of the study plots, heritability estimates dropped towards zero (0.01 ± 0.00). These results confirm that the observed overall habitat heritability was due to philopatric individuals breeding in their natal plot and, thus, to location heritability. The fact that heritability dropped when immigrants were randomized across the study plots reflects the elimination of the parent-offspring link for breeding site vegetation composition. When randomizing the breeding nest box for the majority of the population (71%), the natal box of the philopatric individuals was most likely in a different study plot with a different vegetation, and the vegetation composition of their own breeding box and their natal box was dissimilar. Overall, these results are in concordance with

previous studies that have suggested that early experience in the natal site influences habitat choice in the pied flycatcher (Camacho et al., 2016; Chernetsov et al., 2006).

The subset data analysis and simulations provided no evidence for direct genetic habitat choice (i.e., genetic preference for a specific breeding site vegetation composition) or matching habitat choice. If either of these mechanisms had been present, we would have observed heritability in the subset of non-philopatric individuals too. In the first case, offspring would have inherited their parents' genetic preferences, which would have led them to choose a similar environment to that preferred by their parents. In the second case, because offspring generally inherit a phenotype similar to that of their parents, this would have led them to select a similar environment where they would have optimal local performance. There is no reason to think that only philopatric individuals exert either of these types of habitat choice. However, our study could be extended to test for matching habitat choice by using animal models with the habitat as a dependent variable and phenotypic traits as covariates (Munar-Delgado et al., 2023). In this way it could be possible to test if the phenotypic traits have any effect on habitat choice. For example, body size is thought to affect phenotype-dependent dispersal in pied flycatchers (Camacho et al., 2019) that could directly affect habitat choice.

In our study population, vegetation composition in each plot is relatively more homogeneous than vegetation composition between plots. This is likely why philopatric pied flycatchers inherit breeding site vegetation composition: philopatric individuals inherit the location, and thereby indirectly the vegetation. This is a form of non-genetic inheritance (of the extended phenotype), and results in non-genetic parent-offspring similarity (Bonduriansky et al., 2012).

If so, this contradicts the common assumption in quantitative genetics that the estimated heritability reflects the genetic basis of the trait.

However, the philopatric behavior causing the extended phenotype inheritance was estimated to be heritable itself too. We found a heritability of natal philopatry of 0.27 ± 0.04, which is close to the mean value reported for behavioral traits (0.30 ± 0.03; Stirling et al., 2002) and also close to the observed range for heritability of this trait in the closely related collared flycatcher *Ficedula albicollis* (0.30 ± 0.07 and 0.47 ± 0.10; Doligez et al., 2009; although note that these values could be overestimated due to the parent-offspring regression methodology used, Postma, 2014). In this case, the animal model could in fact be reflecting the genetic basis of philopatry (Munar-Delgado et al. 2023), and thereby a genetic basis to the environment. Here, we did not take into account that dispersal in males pied flycatchers can be dependent on arrival date in the recruitment year (Hušek et al., 2014), on natal brood size (Pärt, 1990), on hatching date (Smith et al., 1989) or natal territory quality (Potti & Montalvo, 1991). These variables could be taken into account to expand the study on the heritability of the environment.

Although the observed heritability of breeding site vegetation composition may be influenced by these various factors, the observation that pied flycatchers (partially) inherit the habitat remains unchanged. Therefore, we repeat that it is possible that for this pied flycatcher population the habitat can evolve to improve the phenotype-environment match.

In conclusion, as a case study we have shown how animal models can be used to estimate the heritability of the environment. This confirms that applying this methodology to other existing

datasets which contain information on relatedness and individual local environments can provide insight into the heritability of the environment, and its consequences for the evolution of extended phenotypes and regular phenotypes alike.

Chapter III: Experimental rapid and small-scale ecological population divergence in the absence of current natural selection

This chapter reproduces entirely the manuscript:

Munar-Delgado, G., Hidalgo-Rodríguez, P., Sánchez-Montes, G., Tella, J.L., Potti, J., Forstmeier, W, Edelaar, P. Experimental rapid and small-scale ecological population divergence in the absence of current natural selection. *In preparation*

Abstract

Decades of research have greatly contributed to our knowledge on adaptation. However, research on adaptive population divergence has mostly focused on natural selection as its driver. Here we show that matching habitat choice, a phenotype-dependent type of habitat choice, can lead to ecological population divergence even in the absence of current natural selection. For this, we used two sets of transponder-operated bird feeders and equipped a population of captive zebra finches with two corresponding sets of transponder-tags. Half of the birds were restricted to only have access to food at feeders placed at one end of the aviary and the other half to only have access at the other end, mimicking a novel ecological challenge and two matching ecological traits. For both males and females, the majority of individuals bred at the end of the aviary where they had access to food, generating a pattern of adaptive population divergence based on the ecological match between transponder and feeder. As a consequence of this spatial self-sorting, most individuals bred with an individual that had access to food at the same location as themselves, generating positive assortative mating for their ecological trait (the tag). Because of this, if we assume the ecological trait was heritable, most fledglings would already be born in their matching local habitat, thereby maintaining the ecological population divergence into the next generation. These results demonstrate the potential for matching habitat choice to drive rapid population divergence and adaptation, especially at small spatial scales. This may be of significant importance for population and species management, especially in the context of ongoing global change associated with novel and rapid environmental change.

Key words: matching habitat choice, assortative mating, ecological divergence, adaptation

Introduction

Immersed in the Anthropocene, species are experiencing a period of unprecedented novel and rapid environmental changes (Waters et al., 2016). This has led to an enormous loss of biodiversity, with extinction rates far above pre-human levels (Johnson et al., 2017). Human activity also results in the spread of invasive alien species, which impact biodiversity, economy and health (Pyšek et al., 2020). In this context of environmental change, one of the biggest challenges in evolutionary ecology is understanding in detail how species and populations adapt to their environment, to improve management and conservation strategies for native species as well as control plans for invasive species (Garant, 2020; Otto, 2018). Experiments on adaptation began more than 70 years ago and have provided much insight (Rice & Hostert, 1993; for recent reviews, Schlichting, 2021; Wadgymar et al. 2022). However, research on adaptation has mostly focused on natural selection and phenotypic plasticity, while other mechanisms that could result in the same outcomes have been relatively neglected (Edelaar & Bolnick, 2019; Trappes et al., 2022). This could be biasing our knowledge and, consequently, our capacity for action.

One alternative mechanism for adaptation is Matching Habitat Choice (MHC). MHC is an ecological process based on individuals' self-assessment of local performance across habitats, followed by settling (or spending more time) where performance is highest (Edelaar et al., 2008). One example of MHC are grasshoppers of different colors which choose to perch on color-matching substrates, thereby increasing their crypsis (Camacho et al., 2020; Edelaar et al., 2019). The choice of settlement is based on the individual's perception of how a given habitat is affecting its phenotype-environment match (e.g., degree of crypsis) and therefore its expected fitness (e.g., lower predation risk). The operation of this habitat selection criterion

was confirmed by experimentally manipulating the grasshopper's color, which caused the individuals to move towards a new color-matching substrate, as predicted under MHC (Camacho et al., 2020; Edelaar et al., 2019). MHC is thereby responsive to an individual's ecological performance (e.g., relative crypsis), and therefore to its phenotype (e.g., its color). In this way, MHC has been hypothesized to influence a large number of phenomena, such as individual and population fitness, local adaptation, maintenance of genetic variation, ecological population divergence, colonization of novel environments, positive assortative mating and even speciation (Berner & Thibert-Plante, 2015; Edelaar et al., 2008; Nicolaus & Edelaar, 2018; Porter & Akcali, 2020; Porter & Benkman, 2022; Ravigné et al., 2009; Scheiner et al., 2022).

It is well-known that habitat choice can cause adaptation (Edelaar et al., 2019; Porter & Akcali, 2020; Richardson et al., 2014). However, the responsiveness of MHC has a distinctive feature with respect to the other two forms of habitat choice, imprinting and genetically determined habitat choice (Akcali & Porter, 2017; Camacho et al., 2015). These two other forms do not depend on an assessment of local performance, and therefore do not respond to variation in the phenotype. For example, grasshoppers could alternatively prefer the habitat they grew up in (imprinting), or prefer the habitat for which they have a genetic preference, both irrespective of their own color. With respect to this flexibility (the choice of habitat depends on the phenotype), the comparison between MHC and the other two forms of habitat choice is therefore similar to the comparison between a trait with or without phenotypic plasticity (except with a reversed causality: the phenotype affects the environment). As a consequence of this lack of responsiveness to phenotypic variation, the other forms of habitat choice do not necessarily lead to an improved phenotype-environment match (e.g., grasshoppers could actually prefer a habitat in which they are less cryptic). In contrast, MHC is expected to create

adaptive phenotype-environment covariance, with individuals with different phenotypes settling in different local habitats, each in the habitat where they perceive to do best. On top of that, if this phenotypic variation has a genetic basis, MHC is expected to also lead to non-random gene flow (Edelaar & Bolnick, 2012) and to create genotype-environment covariance.

Despite the potential ecological and evolutionary implications of MHC and a noticeable rise in interest in it in recent years, the empirical evidence supporting it is still scarce, in part because it is overlooked, in part because it is hard to obtain convincing support that excludes alternative explanations (Edelaar et al., 2019; Edelaar & Bolnick, 2019). Because of this, MHC has only been confirmed to influence a few of the phenomena it has been predicted to cause. Specifically, there has not been any experimental test for whether MHC can drive assortative mating and reproductive isolation (but see Porter & Benkman, 2022 for an observational study). This could arise because the phenotype-environment covariance due to MHC could subsequently and indirectly result in individuals mating with other individuals with a similar phenotype, if mating occurs within the chosen habitat. This is important, as local mating then translates into reduced gene flow between individuals with different phenotypes, maintains genetic variation at the metapopulation level, and could provide an initial step towards speciation (Berner & Thibert-Plante, 2015; Bolnick & Otto, 2013; Edelaar et al., 2019; Nicolaus & Edelaar, 2018). Basically, assortative mating due to MHC means that the population-structuring effect of MHC is not lost during reproduction, and propels this effect into the next generation. A test of whether MHC can cause assortative mating is therefore long overdue.

To test whether MHC could result in population divergence and assortative mating we designed an experiment that allowed us to manipulate local performance (individual food intake rate) across local environments. We created a spatially-structured environment by placing two sets of transponder-operated feeders (NatureCounters) at the two extremes of a 4.6 by 4.2-meter aviary. Individual variation in local performance was achieved by marking a population of captive zebra finches (*Taeniopygia guttata*) with a leg band containing a built-in transponder tag. These transponders allowed half of the individuals to have access to food only at the feeders placed in one area of the aviary (area A), and the other half of the individuals only in the other area (area B). Thus, with the electronic feeders we mimicked the existence of two types of local habitats differing in resources (areas A and B), and with the transponder tags (types A and B) we mimicked the existence of two ecological traits differing in providing access to these resources, each matching only one of the two available resources. As an example of something similar occurring in nature one could think of crossbills (*Loxia curvirostra* complex). These birds feed on seeds from conifer cones and have different bill morphologies (the ecological trait) that match cone morphologies of different tree species (the local habitat), and crossbills are thought to disperse across the mosaic of patches of different conifer species to increase this match and thereby their food intake rate (Benkman, 2017; Porter & Benkman 2022). In this case, we provided a novel environment where birds had to locally evaluate a familiar ecological performance (food intake rate). By providing nest boxes at both areas, we let individuals assess their local performance in both areas and choose in which area to breed. Once breeding, individuals were assigned to nest boxes and breeding partners through behavioral observations. At the end of the experiment, all adults and fledglings were genotyped to assess genetic parentage, allowing us to associate adults with the nests that contained their fledglings at the genetic level.

For theoretical reasons, we predicted that in this experimental setup: (1) individuals will breed in the same area where they have access to food; (2) individuals pair with other individuals with the same transponder type; (3) pairs will be producing more fledglings if they breed in the area where they have access to food; (4) MHC will be stronger at the genetic level, because it will promote extra-pair copulations with individuals that have access to food in the same area; (5) extra-pair copulations will be assortative; (6) produced offspring would be locally matching.

Based on our results, we demonstrate how MHC can easily arise when there are individual differences in local performance, and that it can drive ecological population divergence. This in turn causes reproduction between similar individuals (positive assortative mating), allowing the divergence to be transmitted to the next generation. In contrast to our prediction, breeding in the area where individuals have access to food did not appear to increase their reproductive success. The upshot is that we can exclude currently acting divergent natural selection as the reason for the observed ecological population divergence and subsequent assortative mating.

Material and Methods

Experimental setup

Two experiments were performed. For experiment 1, males and females (N = 70) were obtained from different commercial providers to ensure that there were no pre-established pairs. Their age was unknown and there was a variety in plumage color phenotypes (fully white individuals were avoided since they were hard to sex). Upon arrival in the lab, birds were uniquely color-banded on both legs, with two regular plastic bands on one leg and a colored plastic leg-band

with a passive integrated transponder (PIT)-tag (Eccel Technology Ltd.) with a unique code on the other leg. Thus, individuals could be identified both visually and electronically (by PIT-tag). Males and females were kept visually isolated in two different rooms prior to the experiment for twelve months. For experiment 2 we used young birds only. These birds (N = 103) were the offspring of adults obtained from the same providers as for experiment 1. They were also color-banded, and were kept with adult individuals until they were 42-57 days old to facilitate sexual imprinting (Vos, 1995).

All birds were fed *ad libitum* and their diet consisted of a mixture of Prestige Tropical Finches seeds (Versele-Laga), grit, cuttlefish bone, and tap water (with extra vitamins once a week). When birds were not in the breeding experiment, fresh leaves (of wild plants) and commercial egg food were also provided.

The experimental indoor aviary was 4.60 x 4.16 x 2.50 m (WxLxH), and was divided into four interconnected areas delimited by plastic mesh. Thus, the different areas were not visually isolated (Fig. 5). The two extremes of the aviary were designated as the breeding areas A and B. These areas were equipped with perches, grit, cuttlebone and water stations during the whole period (i.e., acclimation and experimental phases). During the experiment, feeders and nest boxes were also placed in the breeding areas. Several 0.5 x 2.5 m mosquito net strips were placed in the two intermediate areas to increase the difficulty of flying between the two breeding areas. Globally, both areas were mirror images of each other (Fig. 5).

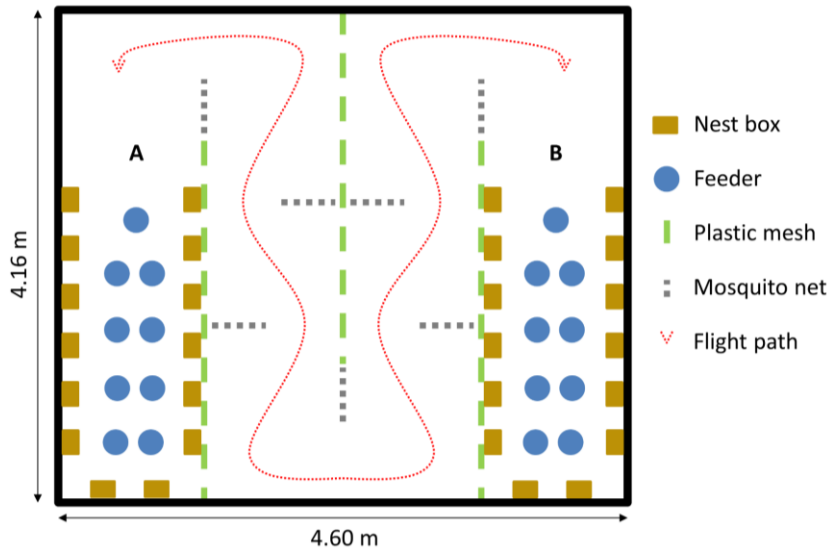

Figure 5. **Schematic representation of the experimental aviary.** A and B denote the name of each breeding area.

Inside this experimental aviary, we created novel environments where we manipulated local ecological performance of the individuals by using 18 electronic radio frequency identification (RFID)-operated bird feeders (NatureCounters). These feeders were able to read and respond to PIT-tags every time a bird perched on them. They could be programmed to remain open or closed. Additionally, when closed, they could be programmed to open and give access to food every time any PIT-tag was read, or only when a specific PIT-tag was read. In this way, each bird could be first familiarized with and then restricted to feed from only one set of feeders. In addition, each time a PIT-tag was read, it was registered along with the time the bird spent on the perch. For the experiments, we created two groups of feeders (corresponding to which of two sets of birds was allowed to feed on them), and placed each group in a different breeding area (either A or B).

We carried out two experiments with some variations. For experiment 1 we used adult male and female birds that were isolated until the beginning of the experiment. Half of the males and

half of the females were randomly assigned to group A (access to the set of feeders in area A) and the other half to group B (access to the other set of feeders in area B). We first introduced the females in the experimental aviary and started the acclimation phase. During this phase, we placed 10 electronic feeders evenly distributed in the two centers of areas A and B and programmed them to stay open, so all birds could have access to the seeds. Once we confirmed that all birds were eating, we programmed the feeders to stay closed until a bird with a PIT-tag approached. In this way, birds got used to the opening mechanism. Then, we started to move the feeders throughout the aviary every 2(±1) days. We first moved all of them to one breeding area of the aviary and then all to the other area. Thus, we ensured that all individuals explored the entire aviary. In the following step, we reprogrammed the feeders so that half of them only gave access to food to birds of group A, and placed them in area A. The other feeders were programmed to only give access to group B, and were placed in area B. Thus, we generated two novel local environments where individuals had a different local ecological performance based on their transponder type, a novel matching ecological trait. Next, for a week, we let them learn by trial and error where each individual had access to food (i.e., let them assess their local performance across local environments). After this, we moved the females out of the experimental aviary, introduced the males and repeated the acclimation procedure previously used for the females. At this point we removed from the experiment 14 individuals that had health issues (N = 7) or that were thought to feed on spilled seeds or on "stolen" seeds from feeders in the area where they were supposed to not have access to food (N = 4), or to balance the male:female ratio after removing the previous birds (N = 3) (see supplementary material for discussion). Thus, 28 males and 28 females stayed in the experiment. After that, we placed 9 feeders in area A and 9 feeders in area B, giving access only to individuals from the corresponding group A or B. Next, we introduced the experienced females with the males in the experimental aviary, and let all individuals move freely. After one week, we placed 28 nest

boxes in the experimental aviary, half at each breeding area, and let the individuals breed. We placed only one nest box per each potential pair so that the entire population could not breed as one colony in only one area. Nonetheless, not all individuals bred and there were a few empty boxes in both areas until the end of both experiments, so nest boxes were not a limiting resource.

In experiment 2, we repeated the procedures used in experiment 1 but used males and females that sexually matured within the experimental aviary and without any separation between the sexes prior to the experiment (i.e., the individuals already grew up in an ecologically heterogeneous environment). To start the acclimation, electronic feeders were placed at both central aviary areas when birds were 15-30 days old. However, conventional feeders were not removed until the age of 33-48 days, and until the age of 42-57 days a few experienced adults with transponders were present to show the young birds how to use the electronic feeders. After the adults were removed, we first moved all the feeders across the experimental aviary and then separated them between both future breeding areas, as for experiment 1. At this point, 33 birds were removed. They either had health issues (N = 3), were not able to use the feeders (N = 6), were thought to feed on spilled seeds or to "steal" seeds from feeders in the area where they were supposed to not have access to food (N = 5), or were removed to improve the male:female ratio after removing the previous birds (N = 19) (see Supplementary Material for discussion). Then, after the remaining individuals had reached sexual maturity (between the age of 92-107 days; males N = 38 and females N = 32), we introduced 34 nest boxes, half at each breeding area, and let the individuals breed.

For both experiments, we started to check nests three weeks after placing the nest boxes, to avoid early disturbance. We checked them once every week until the last fledging had left its nest. For each nestbox, we recorded the number of nestlings. Nestlings were banded at the age of 10±3 days (value ± range).

At nests, identification of breeders was based on color bands and carried out by direct visual observations and camera recordings (network video recorder and 1080P camera, Sannce). For camera recordings, we placed the camera attached to a tripod 50 cm away from the target nest box in such a way that it allowed us to see the color band combination of the individuals entering or leaving the nest box. We then associated breeding individuals with a nest based on individuals' behavior during feeding episodes after young had hatched. We observed that breeding individuals performed nest guarding: one individual stayed inside the nest box until the other breeding individual perched on top of the nest box, in front of the entrance or entered the nest box. Then the guarding individual came out, and the second individual stayed guarding the nest. Zebra finches often (try to) visit other nest boxes. In case the visiting individual was not a breeding individual from the nest, the guarding individual displayed aggressive behavior towards it. Thus, we associated an individual with a nest box and a brood when we observed that a guarding individual let it go inside the nest box without displaying aggressive behavior. We discarded occasional visits from individuals when there was no guarding individual present. Not all breeding pairs were typical male-female pairs, so we classified the breeding individuals as being part of a heterosexual pair, same-sex pair, or trio (when 3 individuals allowed each other to enter the nest).

To terminate each experiment, we removed clutches initiated after the first fledging had left its nest (i.e., removed potential second clutches). Thus, we ensured that each pair had only one clutch. Also, for both experiments, we replaced the electronic feeders with conventional feeders 10 days after the first fledgling left its nest, since fledglings needed to learn feeding by themselves but carried no transponders.

The experiments were performed in installations accredited for animal experiments (number ES410910008004), and approved by the relevant authorities (Consejería de Agricultura, Pesca y Desarrollo Rural of the Junta de Andalucía, permit number 28/03/2018/040). All used adults and produced offspring were returned to the original providers at the end of the study.

Genotyping and parentage analysis

At the end of the experiments, a blood sample was taken and analyzed from all the adults and fledglings involved in the experiments. We did not analyze unhatched eggs and dead nestlings since they were not part of the final population. DNA was isolated using an extraction robot (Freedom EVO 100, Tecan). All DNA samples were genotyped at 10 microsatellite loci (Forstmeier, et al., 2007a) with PCR multiplex cycles consisting of initial denaturation (95°C, 5 min), 23-30 cycles (depending on the multiplex reaction) of denaturation (95°C, 30 s), annealing (59ºC or 60°C) and extension (72°C, 30 s), followed by a final extension step (60°C, 30 min). All reactions were run in a total volume of 10 µl, containing 5 µl of Type-it Master Mix (Qiagen), 1 µl of primer mix, 3 µl of H_2O and 1 µl of DNA (see Table S8). Genotyping was performed on an ABI PRISM 3130 sequencer with the GeneScan 500 LIZ standard. Allele peaks were assigned manually in GeneMapper ver. 4.0.

For parentage analysis we used CERVUS version 3.0.7 (Marshall et al., 1998), which uses a likelihood approach to infer parentage. Parents were assigned on the basis of the highest log-likelihood ratio score (LOD). For each experiment, the simulation was run with the following settings: 10,000 offspring with a candidate number of parents equal to the total number of adult individuals in each experiment; the proportion of sampled candidate parents was 1; the rate loci mistyped was set to 0.01. For each experiment, we ran the analysis without specifying known parents, including all males and females as potential candidate parents.

In this way, we identified all reproductive individuals (genetic parents of fledglings), we associated each fledgling with two reproductive individuals and each reproductive individual was associated with the nest(s) and area(s) where its fledglings had hatched.

Establishment of phenotype-environment matching

Breeding individuals (see criteria above) were classified as matching individuals if they had access to food in the same area where they chose to breed (i.e., a match between transponder type and breeding area). Individuals breeding in the opposite area to their feeding site were classified as non-matching. On the other hand, through the use of genetic parentage analysis, reproductive individual-nest combinations (see above) were classified as matching if the reproductive individual (i.e., genetic parent) had access to food in the same area where the nest was and as non-matching if not. Thus, at the genetic level, reproductive individuals could have be matching and non-matching at the same time if they had genetic offspring in nests in both areas.

We also classified fledglings as matching or not. For this, we assumed that offspring inherit the transponder type from their genetic parents, as follows. If both genetic parents had the same transponder type, offspring inherit the same transponder type as their parents. If genetic parents had different transponders, we assigned each offspring to inherit either transponder type with an equal probability. Finally, we compared the "virtual" inherited transponder type with the side of the aviary where offspring had fledged to classify them as "matching" or "non-matching".

Statistical analysis

All statistical analyses were conducted with R 3.4.1 (R Core Team, ref). GLMs and GLMMs were fitted with functions glm and glmer, respectively, using the package 'lme4' (Bates et al., 2015). For all models, experiment number was included as a fixed effect to account for any differences between experiments (with only two levels, we decided not to fit it as a random effect). We don´t explicitly investigate and discuss differences between experiments, which seem mild at most (suggesting our results are robust with respect to this variation in design). Model predictions and 95% confidence intervals (CI) were obtained with the function 'get_model_data' from the package 'sjPlot' (Lüdecke, 2022). Statistical significance of variables is based on log-likelihood ratio tests comparing models with versus without the tested variable.

Do individuals breed in the same area where they have access to food?

We tested for matching habitat choice using a binomial GLM with breeding area (A = 1; B = 0) as the binary response variable. Fixed factors included male and female transponder type

(A/B). For this model we included data from all breeding pairs with hatchlings, and tested the effect of transponder type for both breeding males and females. We only included data from heterosexual pairs to be able to control for the effect of the opposite sex (trios and same-sex pairs turned out to be relatively common). To simplify the model, we excluded two pairs with a repeated (polygynous) male. On the other hand, to increase sample size, we also tested for matching habitat choice for the whole breeding population, including all individuals involved in same-sex pairs and trios. We fitted breeder ID as a random effect (GLMM) to account for repeated measures for two polygynous males. Fixed effects included transponder type of the breeding individual, sex, and their interaction to test for any differential effect of transponder type between the sexes.

For the models above, we only included unique breeding individual-nest combinations, so not taking into account the number of offspring hatched for each combination. We did this because breeding area selection could be affecting the number of offspring (e.g., having more offspring when breeding at the area where they had access to food), and we preferred to give equal weight to each individual breeder. Also, breeding area selection is not done independently for each offspring (they are typically produced in the same nest), so by focusing on unique individual-nest combinations we avoid inflated statistical significance due to pseudoreplication.

Do individuals pair with individuals with the same transponder type?

To test if the proportion of pairs that bred assortatively for transponder type (i.e., local ecological performance) differed from that expected if mating was random (50% assortative), we used a Chi-square test of expected frequencies. We only included heterosexual pairs to ensure that pairs had not already formed before the beginning of the experiment (males and

females were spatially separated before experiment 1, and might have formed same-sex pair bonds (Adkins-Regan & Krakauer, 2000)). We did not include trios either because if there were two individuals with the same type of transponder and one with a different one, we could not determine if the trio was assortative or not.

Do pairs produce more fledglings if they breed in the area where they have access to food?

We investigated if any matching habitat choice for breeding had an effect on reproductive success (i.e., led to adaptation). For this, we tested whether the number of matching individuals per breeding pair (0, 1 or 2) affected their reproductive success (GLM, with the numberof fledglings as response variable and assuming a Poisson error distribution).

How strong is the matching breeding habitat choice at the genetic level?

We tested for matching breeding habitat choice at the genetic level (i.e., a match between the area where the genetic parent had access to food and where its fledglings hatched) using a binomial GLMM with the breeding area where their fledglings hatched as the response variable. We fitted a separate model for males and females. Fixed factors included individual transponder type (A/B). For both models, we included individual ID as a random effect to account for individuals with fledglings in different nest boxes. After observing the results for the males, we added extra-pair condition (whether a fledgling is raised in that nest due to an extra-pair fertilization or not) and its interaction with male transponder type as fixed effects. To classify genetic males (fathers) as either within-pair or extra-pair, we only included males from heterosexual pairs since we could not be sure about breeding bonds with males in same-sex pairs with two females or in trios. We considered this interaction because the effect of male

transponder type on breeding area appeared to be virtually absent at the genetic level, and we hypothesized that (in contrast to our initial prediction) the effect could be the opposite in extra-pair fertilizations (e.g., males have offspring with their breeding pair in the area where they feed, but have extra-pair offspring at the opposite area). Finally, we tested for matching breeding habitat choice at the genetic level for within-pair males using a binomial GLM with the breeding area where their fledglings hatched as the response variable.

For the models above, we only included unique individual-nest combinations, so not taking into account the number of offspring hatched for each combination. We did this because breeding area selection could be affecting the number of offspring (e.g., having more offspring when breeding at the area where they had access to food), and we preferred to give equal weight to each individual. Also, breeding area selection is not done independently for each offspring (they are typically produced in the same nest), so by focusing on unique individual-nest combinations we avoid inflated statistical significance due to pseudoreplication.

Are within-pair and extra-pair parentage assortative for transponder type?

After identifying extra-pair parentage (see above) we extracted each male-female genetic combination that resulted in within-pair and extra-pair offspring. Then, for both groups, we tested if the proportion of male-female genetic combinations that were assortative for transponder type differed from that expected if extra-pair matings were random (50% assortative). We used a Chi-square test of expected frequencies and, to avoid pseudoreplication, each unique combination was only counted once.

Do the (virtual) phenotypes of the produced fledglings and the entire population match the local environment?

Finally, to test if the proportion of matching fledglings out of all produced fledglings differed from that expected by chance (50% matching), we also used a Chi-square test of expected frequencies. The same test was used to test if the proportion of matching individuals for the entire population (breeding individuals plus fledglings) differed from that expected by chance (50% matching).

Results

Data summary

As is usual for studies on zebra finches, not all individuals established pair bonds (reviewed in Griffith et al. 2017). In addition, some individuals established pair bonds with an individual of the same sex, or with two individuals (Tables 1 and 2 provide a summary of the main raw results on pairing at the social and genetic level, respectively).

Breeders					Non-breeders
68					58
Heterosexual				**Same-sex/trios**	
46[1]				19	
Habitat matching		**Assortative mating**		**Habitat matching**	
Yes	**No**	**Yes**	**No**	**Yes**	**No**
36	10	34	12	14	5

Table 1. Overview of where zebra finches bred, and with whom, as based on visual observations on breeding individuals (raising fledglings in a nest). See Methods for a description of each classification. [1] There were 49 breeding heterosexual individuals, but one polygamous male and its two female partners were excluded to simplify the analysis.

Reproductive individuals			
Female-nest combinations		**Male-nest combinations**	
34		49	
Matching		**Matching**	
Yes	**No**	**Yes**	**No**
25	9	29	20

Table 2. Overview of where female or male zebra finches produced offspring, as based on genetic parentage analysis. See Methods for a description of each classification.

The majority of individuals bred in the same area where they had access to food

For heterosexual pairs (N=23), the probability for 9 males with transponder A to breed in area A was 0.85 [95% confidence interval: 0.38, 0.98], significantly higher than for 14 males with transponder B (0.34 [0.12, 0.65]; LRT: $\chi 2$ (1) = 4.016, p = 0.045). Similarly, for 11 females with transponder A the probability to breed in area A was 0.79 [0.40, 0.96], much higher than for 12 females with transponder B, although the effect was not quite significant (0.33 [0.10, 0.70], LRT: $\chi 2$ (1) = 3.32, p = 0.069; Fig. 1a; Table S1). For the whole population of breeding individuals (including same-sex pairs, trios, and polygynous birds, total N = 68) and controlling for sex and its interaction with transponder type, the probability for individuals with transponder A to breed in area A was 0.86 [0.45, 0.98], significantly higher than for individuals with transponder B (0.19 [0.04, 0.57], LRT: $\chi 2$ (1) = 24.01, p < 0.00001; Fig. 1b; Table S2). The interaction effect between transponder type and sex was not significant (LRT: $\chi 2$ (1) = 0.513, p = 0.474).

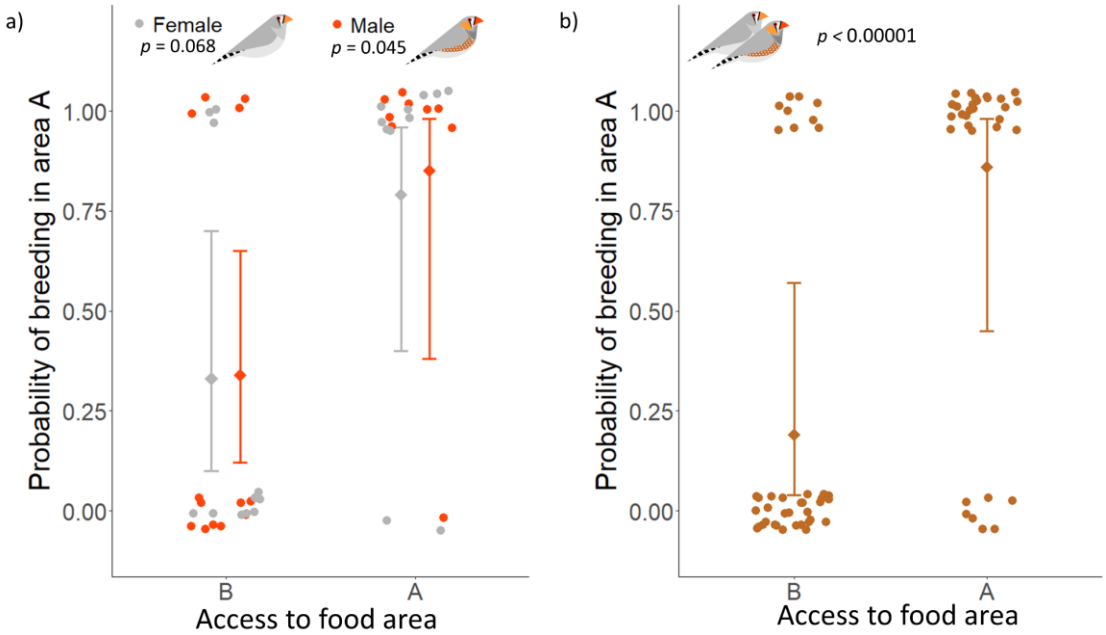

Figure 1. **Matching breeding habitat choice as based on nest location.** a) Model predictions (binomial GLM) for the probability of breeding in area A for females (gray diamonds) and

males (red diamonds) depending on their transponder type, where remaining covariates are set to their means. Error bars represent the 95% CI of model predictions. Dots are raw data for breeding females (gray) and males (red). b) Model predictions (binomial GLMM) for all breeding individuals depending on their transponder type, where remaining covariates are set to their means. Error bars represent the 95% CI of model prediction. Brown dots are raw data.

The majority of individuals paired assortatively for transponder type

We found that 74% of heterosexual pairs (17 out of 23) were assortative for their transponder type ($\chi 2$ (1) = 5.26, p = 0.022) (Fig. 2).

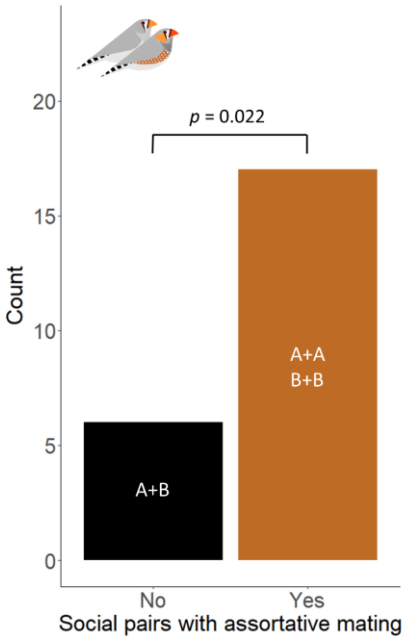

Figure 2. Assortative mating for transponder type with respect to the social mate. Number of observed heterosexual disassortative or assortative breeding pairs (letters indicate the transponder type of the two pair members).

Individuals breeding in their matching area did not have greater reproductive success

For heterosexual pairs (N = 23), there was little evidence suggesting that matching individuals had higher reproductive success (LRT: $\chi 2$ (1) = 0.208, p = 0.648; Fig. 3; Table S3 Supplementary Material).

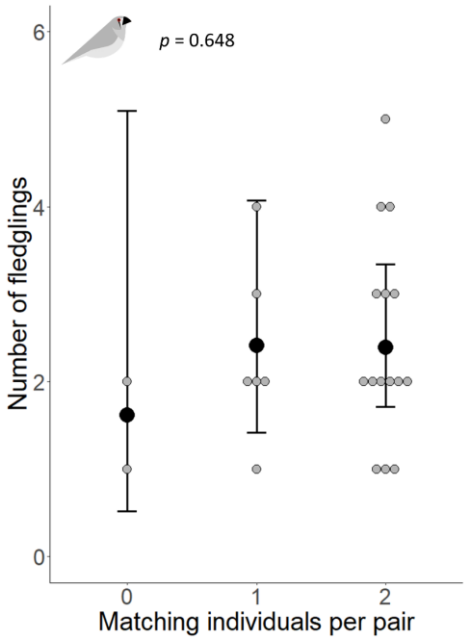

Figure 3. Reproductive success for breeding pairs. Number of fledglings for pairs with 0, 1 or 2 individuals whose transponder matches the breeding area. Black dots represent the model estimates and the error bars their 95% CI. Gray dots represent raw data.

The majority of females produced fledglings in their matching area but males produced them in both

At the genetic level, we identified a total of 34 reproductive female-nest combinations and 49 reproductive male-nest combinations. The probability of females with transponder A of having genetic fledglings in area A was 0.65 [0.36, 0.68] and for females with transponder B this was significantly lower at 0.16 [0.03, 0.58] (LRT: $\chi 2$ (1) = 8.07, p = 0.004; Fig. 4a; Table S4).

However, males with transponder A and transponder B showed no significant difference in their probability of having genetic fledglings in area A, at 0.56 [0.28, 0.81] and 0.34 [0.15, 0.61] respectively (LRT: $\chi 2$ (1) = 1.22, p = 0.268; Fig. 4a; Table S5). We tested if this smaller difference in males was because extra-pair offspring were predominantly produced in one area. Indeed, we observed a significant interaction between transponder type and within-pair/extra-pair male status (LRT: $\chi 2$ (1) = 4.32, p = 0.038; Fig. 4b; Table S6). For within-pair males, the probability for a male with transponder A or B of having offspring in area A was 0.81 [0.25, 0.98] versus 0.21 [0.03, 0.68] respectively. In contrast, the probability for an extra-pair male with transponder A or B of having fledglings in area A was closer to random (and, unexpectedly, even somewhat larger for males with transponder B in area A), at 0.39 [0.09, 0.81] versus 0.55 [0.11, 0.93] respectively. Finally, when tested only for within-pair males (N = 17), the probability of individuals with transponder A of having genetic fledglings in area A was 0.86 [0.42, 0.98] and for individuals with transponder B this was significantly lower at 0.30 [0.10, 0.62] (LRT: $\chi 2$ (1) = 5.65, p = 0.017; Table S7).

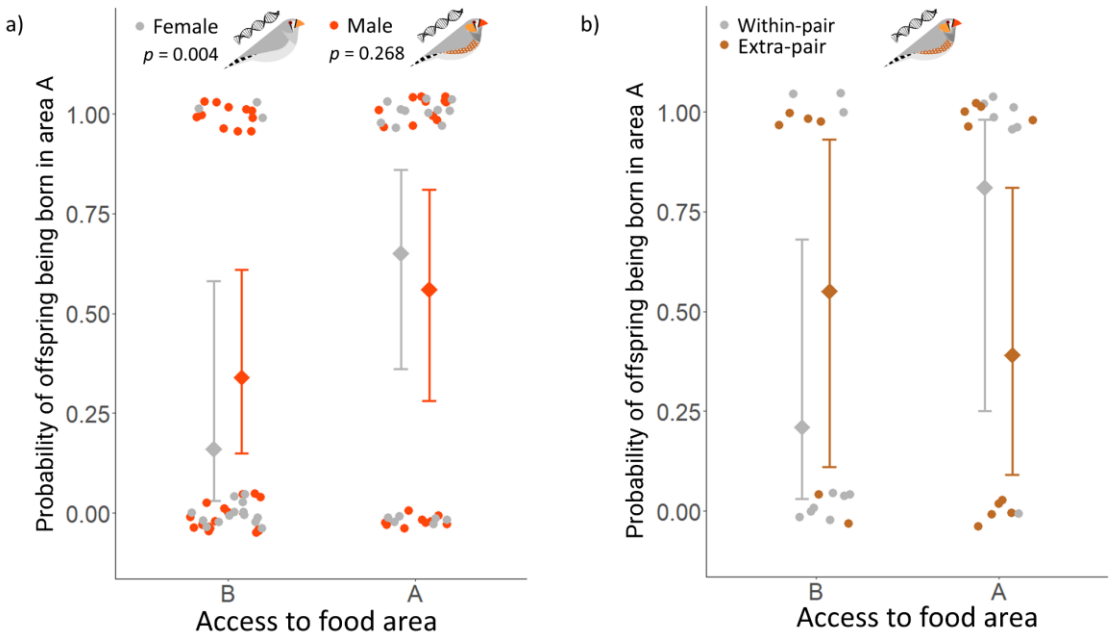

Figure 4. **Matching breeding habitat choice at the genetic level.** a) Model predictions (binomial GLMM) for the probability of having genetic offspring in area A for females (gray diamonds) and males (red diamonds) depending on their transponder type. Remaining covariates are set to their means. Error bars represent the 95% CI of model predictions. Dots are raw data for females (gray) and males (red). b) Model predictions (binomial GLMM) for the probability of having genetic offspring in area A for within-pair males (gray diamonds) and extra-pair males (brown diamonds) depending on their transponder type. Remaining covariates are set to their means. Error bars represent the 95% CI of model predictions. Dots are raw data for within-pair males (gray) and extra-pair males (brown).

Genetic parentage was not assortative for transponder type

We found that 8 out of 16 (50%) unique genetic extra-pair combinations were assortative ($\chi 2$ (1) = 0.00, p = 1.00). The proportion of assortative combinations for within-pair parentage was higher (12 out of 18, 67%), but the difference between the number of assortative and disassortative combinations was not significant ($\chi 2$ (1) = 1.470, p = 0.225).

The majority of the fledgling population as well as the entire population were matching individuals

The number of "virtually" locally matching fledglings (assuming inheritance of parental transponders with random assignment for offspring of disassortative pairs) was higher than expected by chance (53 out of 80, 66%) ($\chi 2$ (1) = 8.45, p = 0.004). Globally, for the entire population (fledglings and breeding individuals, N = 148) there were more matching individuals than expected by chance (105 out of 148, 71%; $\chi 2$ (1) = 25.97, p < 0.000001).

Discussion

Our study provides the first demonstration, to our knowledge, that matching habitat choice (MHC) i) can drive ecological population divergence in a novel environment and be based on a novel trait, and ii) can result in local reproduction, thereby causing assortative mating, which maintains the divergence into the next generation. Together, this suggests that MHC can generally promote adaptive population divergence even in the absence of currently acting divergent natural selection. This possibility directly contradicts the widely held idea that only divergent natural selection can cause adaptive population divergence (e.g., reviewed in Schluter, 2001).

A priori, our predictions as listed in the introduction might have been considered to be unrealistic, since the flight distance between the two feeding areas was only a few meters. In the wild, Zebra finches habitually need to fly several kilometers between feeding and breeding

areas (Zann et al., 1995), such that a few meters would hardly matter. It may also have seemed unrealistic to expect population divergence to arise in a virtually sympatric set up, when, in the wild, populations are genetically undifferentiated over hundreds of kilometers due to very high dispersal rates (Forstmeier et al., 2007b; Zann et al., 1995). Nonetheless, in our experimental setting, zebra finches exposed to a manipulated differential food intake rate across habitats preferentially bred where their food intake rate was higher (although the effect did not quite reach statistical significance in the subset of heterosexual females, possibly because of a limited sample size). Or in other words, MHC arose and individuals had a differential habitat use based on their local ecological performance. This is not because the Zebra finches could not fly more (some actually bred in the non-matching area), but because they did not want to fly more. In the wild, the trade-off between flight distance and food intake will often favor greater flight distances, and therefore reduced population divergence.

It could be argued that our experimental setting is very extreme, since zebra finches could feed at only one of the two areas. An alternative setup could have been to somehow set the feeders to open at different rates in both areas for both bird groups. However, this is not a qualitatively different design, it would only have reduced the magnitude of the effects of MHC. It is also important to note that birds were forced to feed in one of the two areas but they were not forced to breed there (and a few birds indeed chose to breed in their non-matching area). In this way, population divergence was not imposed by setting the feeding trade-off to the largest extent, and our design only maximized its probability of occurrence and its extent (i.e., the power of the experiment to avoid false negatives).

Our results are consistent with predictions from theoretical (Bolnick & Otto, 2013; Edelaar et al., 2008, 2017, 2023; Edelaar & Bolnick, 2012; Mortier et al., 2019; Ravigné et al., 2009;

Scheiner et al., 2022) and empirical studies (Benkman, 2017; Camacho et al., 2020; Edelaar et al., 2019; Holtmann et al., 2017; Porter & Benkman 2022; Regan et al., 2022). Even more, MHC was able to operate in a novel environment and be based on a novel ecological trait. This corroborates that organisms can respond to novel ecological challenges via MHC. In other words, the pre-evolved mechanism of MHC can act in novel environments based on "basic" and generalizable aspects of individuals' ecological performance, such as food intake rate, without having to evolve *de novo* (Edelaar et al., 2008; Edelaar & Bolnick, 2019). This high responsiveness of MHC in novel environments may be particularly relevant under rapid and novel human-induced habitat changes (Sih et al., 2011; Waters et al., 2016), and especially at small spatial and temporal scales where natural selection is unable to achieve divergence as effectively (Richardson et al., 2014). Thus, organisms could rapidly respond to new environmental challenges, even within a single generation, as long as the new local environments affect an aspect of local performance that can somehow be evaluated by the organisms themselves.

We observed ecological population divergence, i.e., phenotype-environment correlation, due to MHC. Our experimental design allows us to exclude alternative explanations for its occurrence (Edelaar & Bolnick, 2019; Trappes et al., 2022). We can exclude imprinting or genetic habitat preference (Akcali & Porter, 2017; Edelaar et al., 2008) since the environments were novel, and individuals were assigned randomly to treatment groups. Phenotypic plasticity is excluded since individuals were not able to modify their transponder type. Adjustment of the environment (cf. Edelaar & Bolnick, 2019) is excluded since individuals were not able to modify (e.g., reprogram or re-engineer) the electronic feeders. Finally, we also prevented divergent natural (survival) selection from acting by not including a few individuals that were not able to learn how to use the electronic feeders (see Methods). This supports the view that

individuals are not just *targets* of selection (i.e., are selected by their environment) but that they can also be *agents* of selection (i.e., actively select their environment) (Edelaar et al., 2019).

Contrary to what we expected, we did not find a significant difference in reproductive success between matching and non-matching individuals (although our sample size is too small to detect weak effects). This might be because the travel distance between feeding areas was very short; longer distances are expected to have (higher) reproductive costs for non-matching individuals. The upshot of this lack of differential reproductive success is that divergent natural selection cannot explain the observed adaptive population divergence.

We confirmed that a non-random mating pattern emerged after manipulating local ecological performance. Most individuals bred with another individual with the same local performance as themselves, thus generating assortative mating for transponder type. This non-random mating could be an indirect effect of MHC (Edelaar et al., 2008; Nicolaus & Edelaar, 2018; Porter & Akcali, 2020; Porter & Benkman, 2022). If zebra finches spent most of the time in their matching area, then most encounters are expected to be between individuals with the same local performance. Therefore, pair bond formation (Maldonado-Chaparro et al., 2021) is also expected to occur between individuals in their matching area and with the same local performance. However, we cannot completely discard additional sources of assortative mating besides MHC. Zebra finches mate assortatively for traits such as neophobia (Pogány et al., 2018) and exploratory behavior (Faust & Goldstein, 2021; Schuett et al., 2011), so it could be possible that they were also able to assess their potential partner's local performance and showed preferences based on that (Snowberg & Benkman, 2009). Because local performance is area specific, the inability of a bird (e.g., of type A) to feed in its non-matching area (B)

could be negatively evaluated by a potential partner of the opposite local performance (B; and vice versa). However, zebra finches show no tendency to pair assortatively for overall phenotypic quality (including past reproductive performance; Wang et al., 2017), so perhaps this is a less likely occurrence.

Contrary to our prediction, transponder type did not affect extra-pair copulations, most likely due to the short distance between the two feeding areas as individuals had to fly only a few meters to change areas and look for extra-pair copulations. As a consequence, male zebra finches had offspring in both local habitats, independent of their own local performance. This could be due to male or female zebra finches looking for extra-pair copulations in their non-matching local habitat, e.g., to avoid mate guarding from their partners (Birkhead et al., 1988) which were feeding in their matching local habitat.

In spite of the frequency of extra-pair matings between birds belonging to opposite groups, if we consider the number of offspring produced by each male-female combination, the effect of MHC on population divergence (assuming virtual inheritance of the transponder type) was maintained in the offspring generations. The simulated inheritance of the transponder type showed that most of the fledged young would have hatched at their matching local habitat, the same habitat where they are expected to breed later as an adult. To the extent that MHC indirectly causes local reproduction, its effects on population structuring are thereby transmitted to the next generation, with its ecological consequences becoming evolutionary effects. In the hypothetical extreme case that individuals respond so strongly to the habitat variation that they all only reproduced in the area where they could feed, then all offspring would be expected to have identical ecological traits to their parents and to later also breed in

the parental habitat, and we would have observed the formation of two ecologically specialized and reproductively fully isolated populations (i.e., biological species) in a single generation due to MHC.

Despite the demonstrated potential relevance of MHC for adaptation, including to aspects of global change, and despite the fact that simulations suggest that in nature it might be as frequent as phenotypic plasticity (Nicolaus & Edelaar, 2018), there are few empirical studies that have tested for its existence in nature and have been able to rule out other processes (e.g., Camacho et al., 2020). To be able to better understand how individuals and populations adapt, and to improve biodiversity management under the scenario of global change, more research should focus on quantifying how important MHC is, and disentangle its potential contribution to divergence and adaptation in natural populations compared to other adaptive processes, under different scenarios and circumstance

Chapter IV: Experimental evidence for performance-dependent movement as an alternative driver of adaptive divergence.

This chapter reproduces entirely the manuscript:

Munar-Delgado, G., Pulido, F., Edelaar, P. Experimental evidence for performance-dependent movement as an alternative driver of adaptive divergence. *In preparation*

Abstract

It is a tenet of evolutionary biology that local adaptation is driven by natural selection, while it is hindered by gene flow. This is because random movements between populations disrupt the local phenotype-environment match. Yet, if individuals move to areas where they expect higher ecological performance, movements between populations could facilitate local adaptation. However, experimental support for this process is still inconclusive. Here we show that performance-dependent movement to the areas where individuals have higher fitness rapidly results in adaptive population divergence. We manipulated local ecological performance in a wild population of Eurasian tree sparrows by creating an artificial ecological trait that gave differential access to a new resource. Individuals exhibited a very strong preference for breeding sites where they had highest ecological performance. This promoted local adaptation, assortative mating and reproductive isolation with respect to the novel trait. Our results experimentally show how local adaptation can be achieved by directed movement, if individuals adaptively select their local environment. Considering this mechanism of adaptation will improve our understanding of how populations and species adapt and diverge. This may be especially relevant for biodiversity management under global change, where organisms face rapid and novel environmental changes.

Key words: matching habitat choice, assortative mating, reproductive isolation, ecological divergence, adaptation

Main

It is a paradigm in evolutionary biology that adaptive population divergence is achieved by divergent natural selection (Futuyma, 2017; Schluter, 2001). Gene flow between populations is thought to hamper this process (Lenormand, 2002; Smadja & Butlin, 2011) as it reduces genetic variation among populations (Aitken & Whitlock, 2013; Fitzpatrick & Reid, 2019). Thus, population isolation is considered a beneficial, if not necessary, condition for adaptive divergence (Harvey et al., 2019; Mayr, 1963). An implicit assumption here is that the individuals that move are a random sample of the population. This, however, may not generally be true. If there is individual variation in local fitness and individuals move to areas where they have higher fitness, then adaptive population divergence will result from a process of spatial self-sorting (Edelaar & Bolnick, 2012; Jacob et al., 2017). This is especially likely if individuals are able to assess their local performance given their phenotype and to move to the area where they perform best, a process called matching habitat choice (Camacho et al., 2020; Edelaar et al., 2008; Ravigne et al., 2004). The best way to demonstrate this process is by manipulating a relevant phenotypic trait (Edelaar et al., 2008), since this changes ecological performance without changing any potentially existing habitat preferences due to imprinting or genetic preference alleles (Akcali & Porter, 2017; Beltman & Metz, 2005; Berner & Thibert-Plante, 2015; Ravigné et al., 2009; Stamps et al., 2009). Ecological population divergence due to this phenotype-dependent movement has been experimentally shown previously (Bolnick et al., 2009; Camacho et al., 2020). However, the fitness consequences of this process have not yet been quantified, and it is unknown whether this ecological population divergence is transmitted to the next generation.

Here we experimentally manipulated local performance of wild Eurasian tree sparrows (*Passer montanus*) by manipulating their ability to feed in different local habitats to test if matching habitat choice can drive adaptive population divergence within and between generations. We developed an experimental system that consisted of passive integrated transponder tags (Fig. 1a; rice-grain size devices emitting unique codes that allow individual identification), representing a novel ecological trait, and programmable feeders (Fig. 1b) with transponder readers (Bonter & Bridge, 2011; Regan et al., 2022), which represent a new food resource. We introduced two groups of feeders in two areas in our study site (Area A and B; Fig. 1c). These provided supplementary food to sparrows ringed with a plastic ring equipped with a transponder tag (Fig. 1a). We programmed the feeders in area A to provide access to food to half of the birds (type A) and the feeders in area B to provide access to the other half (type B). We thereby created the strongest possible trade-off in local performance (Kawecki & Ebert, 2004). The feeders can be seen as two different ecological resources, and the transponder tag as the ecological trait that enable access to either one or the other resource. As an example of a similar phenomenon occurring in nature, one could consider crossbills (*Loxia curvirostra* complex). These birds exhibit different bill morphologies (the ecological trait) that match different cone morphologies of different tree species (the local habitat) and enable differential access to seeds (Benkman, 2017; Porter & Benkman 2022). By generating extreme phenotypes for the tree sparrows, we maximize the costs of mischoosing and enhance matching habitat choice to arise (Camacho & Hendry, 2020). Additionally, nest boxes were placed in both areas and equipped with transponder readers to identify breeding individuals (Fig. 1c). We predicted that if sparrows can assess spatial variation in their ecological performance (i.e. transponder-feeder match) and then breed where their performance is highest, then this transponder-biased movement would drive adaptive population divergence (Edelaar et al., 2008; Edelaar & Bolnick, 2019; Ravigné et al., 2009).

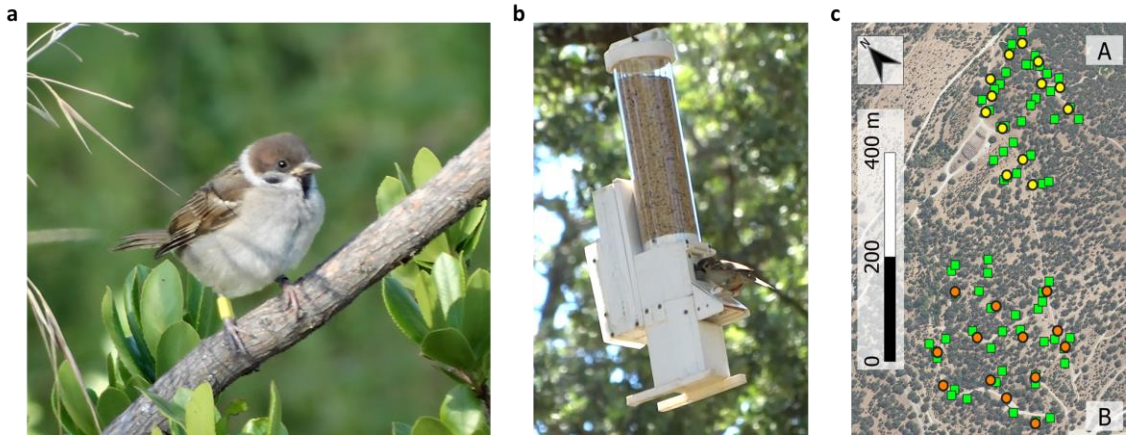

Figure 1. Local performance of wild tree sparrows was manipulated using transponder tags giving access to electronic feeders in only one area. ***a***, A wild tree sparrow with a plastic leg ring equipped with a transponder. **b**, A programmable electronic feeder in the field that allows food access only to specific transponder-tagged birds. **c**, Location of the electronic feeders (yellow and orange points) and of the nest boxes (green squares). Feeders in area A (yellow points) gave access to food to half of the tagged birds (type A); feeders in area B (orange points) gave access to the other half (type B).

Our results confirmed this prediction. For the subset of ringed birds that could compare local performance before selecting their breeding area (n = 41; see Methods), there was a positive association between the area of supplementary food access and the area of breeding (85% of individuals bred in the area where feeders matched their transponder; Fig. 2). An alternative explanation for this observed ecological population divergence might be divergent natural selection (Edelaar & Bolnick, 2019; Schluter, 2001): if home ranges were small, then individuals that had feeders matching their transponder within their home range may have had a higher probability of survival and breeding. However, this explanation can be excluded since we only analyzed birds that were registered visiting feeders in both areas prior to selecting a site for breeding (Methods), i.e., all individuals had access to supplementary food.

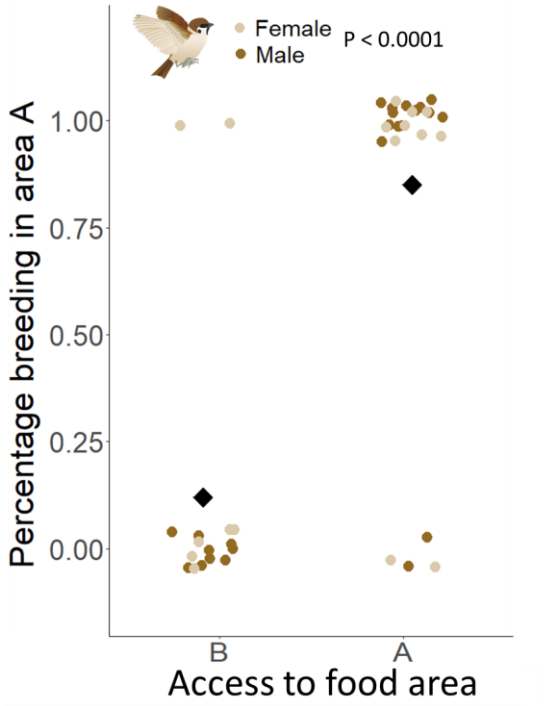

Figure 2. Matching habitat choice generated ecological population divergence. There was a positive association between the area where birds had access to supplementary food and the breeding area (Fisher's exact test, $P < 0.0001$). Dots are raw data for breeding females (tan) and males (brown). Black diamonds are the percentage of individuals with supplementary food access in area B and A that bred in area A.

To investigate whether the observed population divergence was adaptive, we used genetic parentage information (Methods). Individuals that bred in the area where they had access to supplementary food (i.e., feeder-matching individuals) produced more fledglings than those that were mismatched (Fig. 3a). This higher quantity of fledglings could trade-off against lower fledgling quality (Stearns, 1989; Xu et al., 2023), ultimately affecting the parental contribution to the next generation. However, we found no indication that this trade-off reduced the benefit of greater fledgling production by matching individuals (Fig. 3b). Thus, our results show that

the directed movement towards a matching area for breeding resulted in local adaptation despite the small spatial scale of the experimental setup.

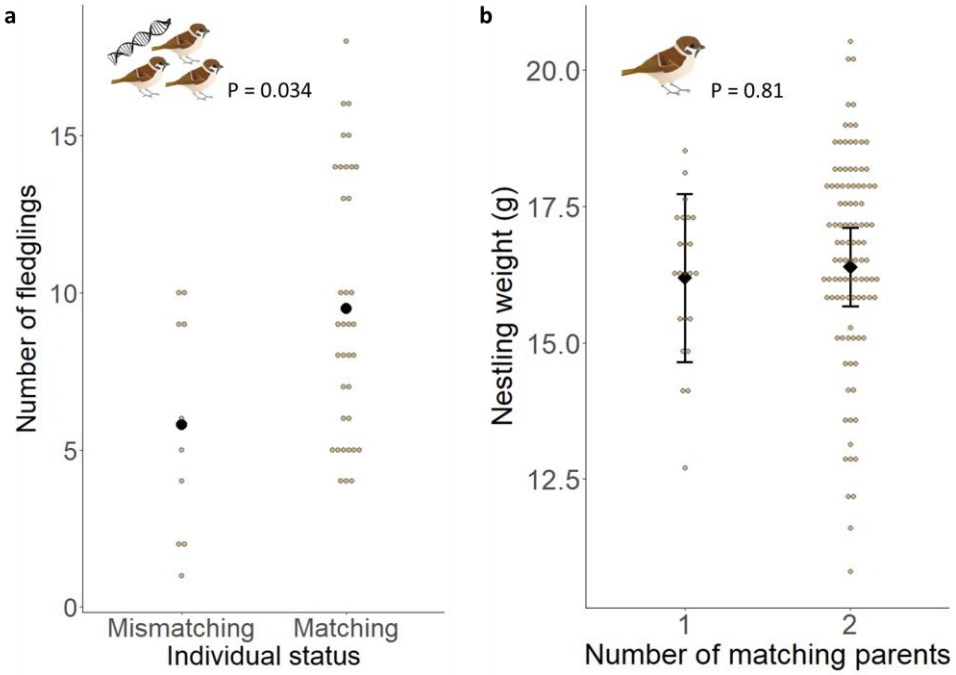

Figure 3. Breeding where the ecological trait matched the ecological resource increased offspring number, while offspring quality was not reduced. a, Number of fledglings (brown points) and population mean (black points) produced by matching versus mismatching individuals (Mann–Whitney U-test, P = 0.034). **b**, Model estimates for mean nestling weight (black diamonds) and raw data for each nestling (brown dots) (Generalized linear mixed model, χ^2 (1) = 0.057, P = 0.81; Extended Data Table 1); because of the strong effect of local performance on the selection of breeding area, there were no pairs composed of two mismatched individuals. Error bars represent the 95% CI of model estimates.

If individuals that share similar ecological traits move to the same area to increase local performance and mate there, this should promote assortative mating for the ecological trait that drives this performance-dependent movement. As a consequence, reproductive isolation between individuals with distinct ecological traits is expected to emerge (Berner & Thibert-Plante, 2015; Bolnick & Otto, 2013; Edelaar & Bolnick, 2019; Nicolaus & Edelaar, 2018; Ravigne et al., 2004; Ravigné et al., 2009). This prediction has hitherto not been tested. To do so, we used the genetic parentage data and focused on genetic pairs where both individuals were tagged. We observed a total of 13 unique genetic mother-father combinations. The majority of them (92%) mated assortatively for area of supplementary food access (Exact binomial test, $P = 0.002$). Five of those mother-father combinations were due to extra-pair copulations, which were all assortative. Hence, no individual bred in one area and had extra-pair copulations in the other area, which would have increased gene flow between the two groups (Baldassarre et al., 2014; Hartman et al., 2012). The I_{PSI} index describes overall reproductive isolation between groups and varies from -1 (maximum disassortative mating) to 1 (maximum assortative mating and complete reproductive isolation) (Rolán-Alvarez & Caballero, 2000). Our two transponder groups had an I_{PSI} value of 0.87, indicating a degree of reproductive isolation that is close to complete.

Reproducing within the habitat where local performance is greater and with a partner with similar ecological traits should result in the transmission of the achieved adaptive population divergence to the next generation, assuming the traits are heritable (Edelaar & Bolnick, 2012; Rundle & Nosil, 2005). To test this, we focused on the 85 fledglings where both genetic parents were part of the experiment (see Methods). We assumed that the fledglings would inherit the transponder type of their parents, which determines their access to the feeders in the area where they were born (see Methods). Our findings show that 90% of the fledglings would have

inherited the type of transponder that gives them access to the feeders in the area where they were born (Fig. 4). This suggests that the adaptive population divergence observed in breeding individuals is likely to be maintained in the next generation.

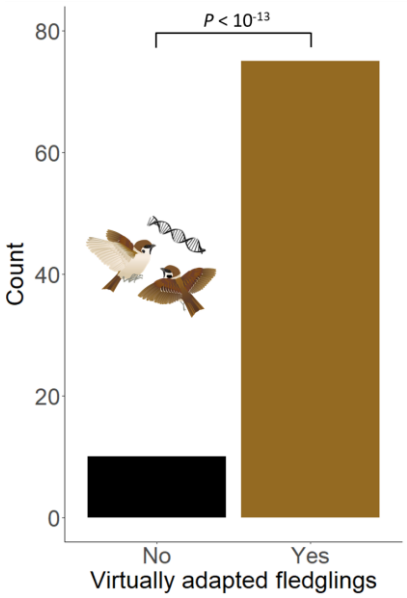

Figure 4. If transponder type was heritable, the majority of fledglings would have been born in the area where they have higher expected fitness. Difference in the number of virtually adapted and not-adapted fledglings (χ^2 (1) = 195.02, P < 10^{-13}).

To our knowledge, we provide the first experimental demonstration that non-random movement driven by differential perception of local performance can promote adaptive population divergence within and between generations. We manipulated a trait (the transponder) which affected local ecological performance (ability to feed), and individuals responded by moving to and breeding where their opportunities for accessing food and their breeding performance were highest (Fig. 2). This created the same pattern expected under divergent natural selection, i.e., that of local adaptation (Edelaar & Bolnick, 2012, p. 20, 2019).

However, current natural selection acting on the sparrows did not cause this pattern; it was caused by selection of the breeding area *by* the sparrows.

Choosing to breed where performance was highest indirectly resulted in assortative mating for the underlying ecological trait (Jiang et al., 2013; Nicolaus & Edelaar, 2018). To the extent that the ecological trait is heritable, this will generate offspring that is locally adapted to their natal site (Fig. 4). Hence, whenever non-random movement affects where reproduction occurs, the adaptive population divergence generated by non-random movement will persist in the next generation.

Non-random movement based on local performance combined with subsequent reproduction after settlement can result in reproductive isolation between populations (Akcali & Porter, 2017; Edelaar et al., 2008; Nicolaus & Edelaar, 2018). Any ecological trait involved in this process could therefore be seen as a 'magic trait' that promotes ecological divergence and reproductive isolation at the same time (Servedio et al., 2011). We used only one ecological trait (the transponder tag) and one aspect of habitat variation (the feeders). When ecological performance depends on multiple aspects of the phenotype and the environment, local reproduction after matching habitat choice would also increase the probability that individuals with different locally adaptive traits mate and produce offspring which combine several adaptive traits (Shine et al., 2011). This could increase variation in local performance across individuals, enhance matching habitat choice, and reinforce adaptive population divergence (Kopp et al., 2018).

Tree sparrows were able to adaptively select breeding areas via self-assessment of local performance based on a novel ecological trait (the transponder tag) and a novel environment (the feeders). Thus, matching habitat choice can be seen as a previously evolved mechanism (under selection itself; Camacho & Hendry, 2020) that allows individuals to rapidly and adaptively respond to novel ecological challenges, as long as they are able to evaluate the effects on their local performance (Edelaar et al., 2008; Edelaar & Bolnick, 2019). Rapid local adaptation could also be achieved by natural selection (Losos et al., 2006). However, strong natural selection is intrinsically linked to high demographic costs (Haldane, 1957), which can lead to a decline in phenotypic and genetic variation, and in population size. This, in turn, can reduce the adaptive potential of a population, increase sensitivity to stochastic events, and ultimately lead to extinction (Orr et al., 2008). In contrast, performance-dependent movement can protect genetic variation and reduce demographic costs, since pre-adapted individuals are using or colonizing different environments (Edelaar et al., 2008; Ravigne et al., 2004; Ravigné et al., 2009). Recognizing these consequences may be relevant for management and protection of biodiversity as organisms are increasingly exposed to rapid and novel environmental challenges as part of global change (Dirzo et al., 2014; Thomas et al., 2004b).

Methods

Study population and site

The study was conducted in a population of tree sparrows breeding in nest boxes in the Encinar de San Pedro reserve in Madrid, Spain (40°25′34″N 3°45′14″W). Tree sparrows were captured in the area during 2020-2022 using three different methods: (i) periodic mistnetting throughout the year, (ii) trapping nestlings and breeding adults in nest boxes during the breeding season,

and (iii) trapping individuals in nest boxes while roosting at night in winter. Each bird was marked with a numbered metal leg ring and a plastic leg ring with a passive integrated transponder (PIT)-tag with a unique identification code (Eccel Technology Ltd.). Additionally, a small blood sample (<100 µl) was collected from the brachial vein in nestlings and the jugular vein in adults for molecular parentage analysis.

Programmable feeders and nest boxes equipped with transponder readers

We used programmable electronic radio frequency identification (RFID)-operated feeders (NatureCounters) to manipulate access of PIT-tagged individuals to supplementary food. These feeders were able to read the PIT-tags when a tagged bird perched on the feeder and to respond according to 3 programmable modes. In mode 1, feeders were open by default, giving access to food to all birds (including other species). In mode 2, feeders were closed by default, and opened only when a PIT-tagged tree sparrow perched on it. In mode 3, feeders were closed by default and opened only when birds with feeder-matching PIT-tags (i.e., specific identification codes) perched on the feeder. Additionally, for all birds equipped with a PIT-tag the feeders registered each visit (including date and time) and individual identity. Feeders were filled with a mixture of Prestige Tropical Finches seeds (Versele-Laga) such that tree sparrows could feed *ad libitum*. A small container below the feeder, and a wire grid on the soil underneath the feeder, largely prevented birds from feeding on spilled seeds.

We used nest boxes that were installed at the study site in previous years for population monitoring purposes (Extended data Fig. 2a). During the experiment, each nest box was equipped with a wooden frontal panel that allowed placement of an electronic RFID-reader

(NatureCounters). The electronic readers registered each bird visit to the nest box and individual identity if a bird was PIT-tagged.

Experimental procedure

In October 2020, the 68 boxes present in the study area were relocated, placing half of them in area A and the other half in area B (Extended data Fig. 2b) and wooden front panels with readers were installed. In June 2021, before the end of the breeding season, we positioned 22 feeders at the study site, 11 in area A and 11 in area B (Fig. 1c). Feeders were set to mode 1 (open by default) so all adults and new fledglings were afforded access to them. In August 2021, after all chicks had fledged, we set all feeders to mode 2 (closed by default, and only opened when a PIT-tagged bird perched on them) so the birds get used to the open-and-close mechanism (a small perspex plate moving up and down). At the same time, we started to move all feeders towards the intermediate zone between both areas to ensure that birds from both areas tracked the feeders and moved across the study area. We moved the feeders every 2-3 days (value ± range), 7 times, 37±20 m (value ± range) each time (Extended Document 1; Supplementary video 1). Once all the feeders were located in the central area, we checked the identity of every PIT-tagged bird that had used them and randomly assigned half of those tagged birds to type A and the other half to type B (Supplementary video 1). Additionally, we randomly assigned all PIT-tagged birds that were not registered by the feeders to either type A or B. This was done in case they returned to the study site later in the season, ensuring that they were already assigned to a type. Thereafter, we set all feeders to mode 3, so that half of them (feeders A) only allowed access to birds with PIT-tag type A, and the other half (feeders B) to birds with PIT-tag type B. In this way, and from this moment on, we manipulated the feeding ability (local performance) of each bird for the two feeder groups. After one week, we moved

the feeders to the experimental areas: feeders A to area A, and feeders B to area B. We moved the feeders every 2±1 days, 7 times in total, with each move being 37±20 m until they reached their final position (Extended Document 1; Supplementary video 1). As a result, by September 2021 we had established two distinct areas (A and B), both with available nest boxes but with different feeders (A or B), where PIT-tagged tree sparrows had differential access to supplementary food (Fig. 1b). This movement of feeders was done directly after the breeding season, because some tree sparrows can select a nest box and start building their nests in autumn (Pielowski & Pinowski, 1962). We maintained the feeders in this mode and position for almost a year (until August 2022), when the breeding season had ended and all experimental data had been collected.

Data collection

From February to August 2022, we checked nest boxes every week to monitor breeding activity. Once we found the first eggs, we placed an electronic reader inside the front panel of that nest box to verify the breeding individuals' identity. When hatchlings were observed, we accurately estimated their age in days based on identification key elaborated by the research group in previous years, with the hatching date set as day 0. On days 9-10, we counted the number of nestlings, ringed them individually, measured their weight, and took a blood sample for parentage analysis. We did not check the nest box for another 10 days to minimize disturbance to the fledglings and avoid early fledging. After this period, we determined the number of fledglings by checking the nest box for any dead individuals. We found that all ringed nestlings had successfully fledged. Although nestlings typically fledge at around day 13-15, shortly after the last inspection, it is possible that some nests may have been predated

before that time. However, here we assumed that the matching status of the parents did not have an effect on predation rate.

Genetic analysis

All DNA samples were genotyped at 9 microsatellite loci (Extended Data Table 2). DNA was extracted from blood samples using the QIAGEN DNeasy Blood & Tissue Kit according to the protocol purification of total DNA from Animal Blood (Qiagen), but lysing cells overnight, and digesting with RNase before the DNA purification. DNA elution was performed in 200 µl of AE buffer. The DNA was quantified using a NanoDrop™, obtaining a range between 6-78 ng/µl.

PCR amplifications were performed by two multiplex PCRs sets, previously designed in silico using the software Multiplex manager (Holleley & Geerts, 2009) and Autodimer (Vallone & Butler, 2004). Multiplex PCR reactions were performed in a Veriti™ thermal cycler (Applied Biosystems). PCR cycling conditions were as follows: an initial denaturation step at 95 °C for 5 min, followed by 30 cycles of denaturation at 95 °C for 30 s, annealing at 56 °C for 45 s and extension at 72 °C for 30 s, and a final extension at 60 °C for 30 min. Multiplex PCRs containing 1× Qiagen Multiplex PCR Master Mix, a final primer concentration between 0.3-0.8 µM of both forward and reverse primers (Extended Data Table 2), 3 µl of template DNA diluted 1/10 and nuclease-free water to a final volume of 10 µl. The forward primers were fluorescently labeled at the 5′-end with HEX, TAMRA, ROX and 6-FAM dyes (Extended Data Table 2), and the reverse primers were 5′-end tagged (5′-GTTTCTT-3′) (Brownstein et al., 1996). PCR products were separated on an ABI 3730 DNA Analyser, adding GS500-LIZ size standard. Electropherograms were automatically scored using GENEMAPPER software v 4.0 (Applied Biosystems) with the created bins, and posteriorly reviewed by eye.

Parentage analysis

We used 8 of the genotyped microsatellites (discarting Pamo1 due to amplification errors) for parentage analysis. We used the software CERVUS version 3.0.7 (Marshall et al., 1998), which uses a likelihood approach to infer parentage. Parents were assigned on the basis of the highest log-likelihood ratio score using the parent pair-sex known option. As candidate parents we included all genotyped individuals that were registered at the feeders during the breeding season. We also included individuals not registered in our nest boxes to account for extra-pair copulations. Nonetheless, the genetic parents of all the observed extra-pair offspring had been recorded breeding in our nest boxes.

The simulation was run with the following settings: 10,000 offspring with a candidate number of parents equal to the total number of adult individuals genotyped and registered in the feeders and/or the readers during the breeding season (39 males and 43 females); the proportion of sampled candidate parents was set to 0.8; the rate loci mistyped was set to 0.01. We ran the analysis without specifying known parents, including all males and females as potential candidate parents.

In this way, we identified the genetic parents of all fledglings that had both parents tagged and with the experimental treatment successfully applied.

Data analysis

All statistical analyses were conducted with R 3.4.1 (R Core Team, 2020). Generalized linear mixed models were fitted with the glmer function of the 'lme4' package (Bates et al., 2015). Model predictions and 95% confidence intervals were obtained with the function 'get_model_data' from the package 'sjPlot' (Lüdecke, 2021).

We first determined which PIT-tagged individuals with differential food-access restrictions had bred in our nest boxes by checking the readers' registers. Next, we identified the individuals to which the experimental treatment was successfully applied. Matching habitat choice requires individuals to assess and compare their local performance across different habitats. To test whether this essential prerequisite was fulfilled for each bird, we checked all feeders' registers to determine if the focal individual attempted to access supplementary food in feeders located in both areas (i.e., both types of feeders) between the day the feeders had been set in their final position in September 2021, and 30 days before its nestlings hatched. We considered the application of the treatment successful when a PIT-tagged bird was registered on feeders from both areas during this time period. When birds were not registered by any feeder, or when they were registered by feeders from one area only, we considered the treatment as unsuccessful. This criterion ensured that all birds considered had the opportunity to assess their local performance across both areas before choosing their breeding area. Note that this is a conservative approach, because individuals that had already evaluated their local performance before the feeders were in their final position, and in response avoided the feeders that did not provide access, are excluded.

To test for the effect of transponder type on the selection of breeding area (i.e., for matching habitat choice) and on ecological population divergence, we considered only individuals where the treatment was successful. To test for performance-dependent selection of breeding area, we used Fisher’s exact test to determine if there was an association between transponder type and breeding area (i.e., the spatial outcome of matching habitat choice).

To investigate the adaptive component of matching habitat choice, we analyzed all PIT-tagged individuals with differential food access in both areas. While some of these individuals did not compare their local performance between the two areas prior to breeding (i.e., the treatment was not applicable for their selection of breeding area), they did have access to the feeders and used them while breeding, so we included them to test if breeding in the feeder-matching area had adaptive effects. We compared the number of fledglings produced by matching individuals (i.e., those that bred in the area where they had access to supplementary food) and mismatching individuals (i.e., those that bred in an area where they did not have access to supplementary food). To take into account extra-pair fertilizations in estimates of total offspring production, we used the genetic parentage data to estimate the number of intra-pair and extra-pair fledglings from each individual. We then used a Mann-Whitney U-test to test for differences in the number of fledglings produced by matching and mismatching individuals.

An increase in the number of fledglings could have negatively affected their quality by reducing their weight and subsequent survival. To investigate this, we used a general linear mixed model to compare the weight of nestlings raised by matching and mismatching individuals. Nestling weight was the response variable, and the number of matching parents in the parents' pair was the predictor variable. Due to the strong effect of matching habitat choice, we observed no pairs with two mismatching parents. We fitted Brood ID as a random effect to account for potential variation between them and for pseudoreplication.

We investigated assortative mating by focusing on pairs where both parents successfully received the experimental treatment. We excluded pairs (genetic father and mother) involving non-tagged individuals, as they were not part of the experiment. We also excluded genetic pairs

with individuals who did not undergo the experimental treatment, as any observed assortative mating in such pairs could not be unequivocally attributed to the effects of spatial sorting due to matching habitat choice. This approach is conservative, but it ensured that any observed assortative mating resulted from matching habitat choice. We defined a genetic pair as assortative when both individuals has the same transponder type, and as disassortative when they had transponders from different groups. We then used a Chi-square test of expected frequencies to determine if the proportion of assortatively mated genetic pairs differed from that expected if mating occurred randomly (i.e., 50% assortative).

We tested for reproductive isolation between both groups of birds (A and B) using the same subset as for estimating assortative mating. We calculated the I_{PSI} index that is based on Pair-Sexual isolation index (PSI) coefficients (Extended data Table X). It describes overall sexual isolation and varies from -1 to 1, with -1 reflecting fully disassortative mating, 0 random mating, and 1 fully assortative mating and complete sexual isolation. This statistic takes into account the proportion of each type of observed mating pair combination (maleA-femaleA, maleA-femaleB, maleB-femaleA and maleB-femaleB) relative to the total number of mating pairs.

We used the parentage data to identify the fledglings that were produced by pairs of adults that both successfully received the experimental treatment for studying the inheritance of ecological population divergence. To this aim, we assumed the inheritance of the transponder type. We assumed that if both genetic parents were in the same type (i.e., assortative mating (Jiang et al., 2013)), their offspring would virtually inherit the same transponder type. If parents were from different types (i.e., disassortative mating), fledgling inherited one of the two types. There were

just 10 fledglings from disassortative parents that were born in area A, so 5 of them inherited type A and 5 type B. Then we compared their virtual transponder type with the area where they were born, to check if they would have been born in the area where they would have had access to supplementary food (locally adapted) or not (not adapted). We used a chi-square test of expected frequencies to determine if the proportion of offspring that is locally adapted differed from what was expected if location of breeding and subsequent mating occurred randomly (i.e., 50% of adapted offspring).

General discussion

General discussion

Using a multi-pronged approach that included simulations to generate extensive datasets, quantitative genetic animal models, mathematical approximations, analyses of long-term data sets from natural populations, experiments with captive and wild birds, automated and observational data collection, and parentage analysis, we obtained different results that contributed to the successful achievement of the objective of this PhD thesis. In the following discussion, I will follow the previously stated subdivision of this work into two blocks, and discuss these results in an integrative manner

The chosen habitat as an extended phenotype

Evolutionary ecology aims to understand how populations adapt and evolve. This usually refers to understanding how environments shape the phenotypes of a population, via natural selection, in order to increase the environment-phenotype match (Darwin, 1859; Schluter, 2001). Experiments aimed at disentangling this process began more than 70 years ago and have had an important impact on evolutionary theory by providing vast insights into how populations respond to environmental pressures (Rice & Hostert, 1993; Schlichting, 2021; Wadgymar et al., 2022) . However the fact that individuals can also affect the environment they experience to increase their own adaptation, by either adjusting or selecting it, has been relatively neglected, and there is still no consensus on how these processes fit into evolutionary theory (Laland et al., 2016; Odling-Smee et al., 2003; Edelaar et al., 2023). To illustrate this point, consider the following example. It is widely accepted among modern biologists that both genes and the environment are responsible for variation of morphological, behavioral, physiological, and life-history traits among individuals, populations, and species. However, the idea that

genes are (partially) responsible for the variation of the environment would be shocking not only to biologists from more outlying fields, but also to many evolutionary biologists and evolutionary ecologists. This notion may be less startling if I clarify that the variation of genes in a population is not responsible for *all* variation of the environment, but it partially contributes to the variation of the environment that individuals experience. Similarly, not *all* variation of the environment is (partially) responsible for the variation of traits in a population, only the environmental variation that individuals experience (this usually does not need to be clarified).

This classical view of unidirectional effects of the environment on phenotypic traits in adaptation terms is also present in the field of quantitative genetics. The majority of research in this field has focused on estimating the heritability of phenotypic traits by disentangling the proportion of variation due to genetic versus environmental variation (but see Gervais et al., 2020, 2022; Järvinen et al., 2017, and Møller, 2006 for exceptions). However, the potential effects of individuals on selecting or adjusting their local environment to improve their fitness have mostly not been taken into account. An exception is the study of indirect genetic effects, when other conspecifics are considered as environment and their phenotype affects the expression of the focal individual phenotype (Bijma, 2014). The second neglected key subject in this field and in evolutionary ecology is the fact that if the selected or adjusted environment has effects on individual fitness and is heritable, the environment that individuals experience can be seen as an integral part of the organism with its own evolutionary dynamics.

Here, we have characterized the local environment affected by the individual (either by selection or adjustment) as an extended phenotype and have justified theoretically and statistically that it can be treated similar to a phenotypic trait when estimating its heritability.

We also show the consequences of not taking into account that the extended phenotype can be heritable when estimating the heritability of other phenotypic traits.

In **Chapter I,** we discussed that while one could argue that an extended phenotype is only an environmental by-product of other relevant phenotypic traits expressed in the organism (e.g., preferences), when investigating the heritability of the environment as an extended phenotype, the appropriate focal trait is the extended phenotype itself. The same is usually done when estimating the heritability of any other phenotypic trait that depends on the expression of other traits (Villemereuil et al., 2018). (Consequently, since in **Chapter II** we were interested in estimating the heritability of the vegetation composition around the breeding nest box (breeding site vegetation composition) for a population of pied flycatchers, we firstly focused on that trait (treated as an extended phenotype) to estimate its heritability.)

We continued **Chapter I,** by using simulations to generate extensive datasets of populations with relatedness information, where we simulated the genetic basis for a phenotypic trait, the genetic basis for the focal environment (extended phenotype), phenotypic plasticity (the effect of the extended phenotype on the phenotypic trait), and/or environmental plasticity (the effect of the phenotypic trait on the extended phenotype). Then, we applied animal models with different causal structures, recognizing the potential bidirectional causality of the phenotype and environment affecting each other. By doing this, we tested the potential application of animal models to estimate the heritability of the environment, the environmental plasticity effects, and the consequences of misinterpreting the causal actions between the local environment and the phenotypic trait. The first result was that by fitting the extended phenotype in the animal model as a dependent variable, it is possible to estimate its heritability. In **Chapter II**, we verified that this finding from **Chapter I** can be extrapolated to datasets from

natural populations. To do so, we used the pied flycatcher data set that contained information about relatedness of individuals and the vegetation composition of their breeding site as a dependent variable. We fitted the breeding-site vegetation composition as a dependent variable, and we were able to estimate its heritability.

The successful development of this methodology facilitates its application to other existing datasets that contain information on relatedness and individual local environments. This is relevant, because when the phenotype-extended phenotype interactions affect individual fitness, the extended phenotype is expected to evolve to improve its match with the organism phenotype (Olding-Smee et al., 2003; Edelaar et al., 2023). Thus, this approach can provide valuable insights into the heritability of the environment and its evolution. Moreover, we were also able to identify the mechanism that caused the habitat inheritance by applying the animal model to different data subsets. This approach could also be extrapolated to other datasets from natural populations.

Additionally, in **Chapter I,** we showed that when the extended phenotype is heritable and it affects a phenotypic trait via phenotypic plasticity, the heritability estimate of the phenotype also increases. This effect is removed when the environment is fitted in the animal model as a covariate. Since this is a common practice in quantitative genetics when using animal models (Wilson et al., 2010), researchers should be aware of this to avoid misinterpretations of their estimates. Thus, taking into account that the focal environment could be heritable, and treating it like an extended phenotype, should help to improve the use of the animal model when estimating heritabilities for wild populations.

In **Chapter I,** we also demonstrated how animal models can be used to estimate the strength of environmental plasticity, which refers to the effect of the phenotypic trait on the local

environment. This can be achieved by fitting the focal environment as a dependent variable and the phenotypic trait as its covariate, in the same way as for phenotypic plasticity but reversing the dependent and independent variables. To our knowledge, this approach has not been previously applied in the literature using animal models. By doing so, we can gain insight into the relatively neglected causal path of the phenotypic trait affecting the environment. For instance, it can be used to investigate how specific phenotypic traits affect the selection of the environment via matching habitat choice, as shown in **Chapters III** and **IV**, or to estimate how they modify the environment via niche construction (Laland et al., 2016). Finally, we showed how assuming a wrong causal structure between the environment and the phenotypic trait when fitting the animal model can result in erroneous heritability estimates; to the extent that in some cases, genetic variation was estimated to be present, when actually there was none. This highlights the importance of correctly identifying the causal relationship between local environments and phenotypic traits to be able to fully understand the evolutionary dynamics of both. Reanalysis of published data sets would be needed to provide insight into how big of a problem this may have been.

Matching habitat choice

"I already knew the concept and it makes sense. [...] It's something that might be there, in nature, but since it's not possible to test or measure it…" is what I was once told by a senior researcher, during a coffee break, in an evolutionary ecology conference after I gave a talk about my results from **Chapter III**. I guess his intention was to congratulate me on my talk but it also felt like he was trying to say that I (and all the previous work) was not able to empirically convince him about the relevance of MHC in nature. So he will not take it into consideration in his study system where the observed population ecological divergence is instead fully attributed to extremely strong divergent natural selection. Considering the trade-offs between

neglecting or including MHC in the experimental evolutionary framework, the balance seems to tilt towards neglecting it. But is this because MHC is not relevant enough, or is it because its relevance is not yet known?

More than fifty years have passed since the process was first described by Fretwell (1969) as ‘adaptive habitat selection’, twenty since Revigné et al. (2004) coined the term ‘matching habitat choice’, and almost fifteen years since Edelaar et al. (2008) unified the terminology used to describe this process, and synthesized its potential ecological and evolutionary consequences. Since then, simulation studies have consistently shown that MHC should be a prevalent mechanism in nature (e.g., Edelaar et al., 2017; Enfjäll & Leimar, 2009; Holtmann et al., 2017; Mortier et al., 2019; Nicolaus & Edelaar, 2018; Nonaka et al., 2015; Pellerin et al., 2019). However, to our knowledge, fewer than twenty papers have focused on MHC (Benkman, 2017; Bolnick et al., 2009; Boyle & Start, 2020; Camacho et al., 2015, 2016, 2020; Camacho & Hendry, 2020; Dreiss et al., 2012; Edelaar et al., 2019; Gillis, 1982; Green et al., 2019; Holtmann et al., 2017; Jacob et al., 2017; Karpestam et al., 2012; Lowe & Addis, 2019; Regan et al., 2022), and they differ in their results and caveats (e.g., not being able to discard other types of habitat choice). Moreover, none of them have been able to experimentally test all the potential ecological and evolutionary consequences of MHC. Due to this limited knowledge about the mechanism, the actual prevalence and relevance of MHC in nature is still unknown. Therefore, while MHC is starting to be recognized as a potential alternative mechanism that could explain or facilitate some of the ecological and evolutionary outcomes observed in nature (e.g., Cheek et al., 2022; Chyb et al., 2021), its consideration remains largely anecdotal, as it is usually not experimentally tested and, if at all, it is just mentioned as a hypothetical alternative. Here, we experimentally confirmed a number of ecological and

evolutionary consequences of MHC that, in previous theoretical work, have been hypothesized to occur - some of them we corroborated for the first time.

Starting with the basics, in **Chapters III** and **IV**, we confirmed the existence of the MHC mechanism. This may not be immediately obvious even after fifty years of research. By manipulating the feeding ability of individuals in different local environments, we were able to observe the emergence of MHC. Individuals were able to assess their local performance in different environments and settle where it was highest, resulting in rapid spatial self-sorting and ecological population divergence (Edelaar et al., 2008). In both chapters, we observed locally adapted subpopulations based on the transponder-feeder match, which generated phenotype-environment covariance. MHC arose within a single generation and acted on a very small spatial scale where natural selection is unable to achieve divergence as effectively (Richardson et al., 2014). In **Chapter III**, both breeding areas were only a few meters apart, while zebra finches are known to fly several kilometers from breeding to feeding areas (Zann et al., 1995). When such ecological population divergence is observed in nature, it is often attributed to the past action of divergent natural selection, which removes mismatched individuals (Schluter, 2001). In contrast, we were able to discard divergent natural selection acting in experiments of **Chapters III** and **IV**. We could similarly discard any other type of habitat choice mechanism (plastic or direct genetic), and MHC was the only plausible explanation for the observed pattern. Thus, individuals are not only passive *subjects* of natural selection, but they can also be *agents* of selection (of the environment), thereby, actively improving their fitness (Edelaar et al., 2019).

It could be argued that our experimental setting in **Chapter III** is very extreme, since zebra finches could feed in only one of the two areas. It is easier to think about less extreme

phenotypic and environmental gradients in nature, but those would reduce the strength of effects, and occurrence of MHC. As proof of principle, in our design we maximized its probability of occurrence and its extent. Moreover, birds were forced to feed in one of the two areas, but they were not forced to breed there. A few birds indeed chose to breed in their non-matching area without negative reproductive consequences. This extreme effect on local performance was reduced in the experiment of **Chapter IV**. Given that tree sparrows had been breeding in the area during previous years without supplemental food, and that non-tagged individuals without access to our feeders bred in our nest boxes during the experiment, tagged birds really had the option to use or not use the feeders, because there was alternative food available in the area. However, they chose to use the feeders and then bred in the area where they had access to supplementary food and where their local performance was higher. Thus, MHC occurrence could also be common when the phenotype-environment match is not as extreme as it was in **Chapter III**.

MHC might also appear to be rare because we are not considering certain scenarios when it might be seen as acting. MHC could be very common in nature if competitive ability is considered as an ecological trait (Fokkema et al., 2021). MHC would generate ecological divergence based on individual-habitat quality (Akcali & Porter, 2017). A 'low quality' individual could have a better local performance in a 'low quality' patch if that implies avoiding competition over a 'high quality' patch with 'high quality' individuals and could, consequently, choose to move there in order to improve fitness. MHC might also be operating when there are species-specific traits that match with specific habitats (Holt & Barfield, 2008). For example, in nature all tree sparrows could choose to move to patches with grass because their food intake is highest there. We tend to see this as given, or as due to a genetic preference, but perhaps sparrows inspect environments, and each individual independently comes to the same

conclusion, that patches with grass isbest for them given their feeding efficiency. In this case, species-specific habitat preference would actually be due to MHC. Hence, MHC might be much more common than it appears to be.

The experimental confirmation that MHC may be the primary mechanism driving ecological population divergence is not new. For example, Edelaar et al. (2019) and Camacho et al. (2020) have already shown how MHC causes ecological divergence in a population of ground-perching grasshoppers. Individuals move towards substrates that better match their body color to improve crypsis. Crypsis presumably reduces mortality, and thus, the adaptive component of MHC seemed obvious (Merilaita & Stevens, 2011). However, they did not observe any differences in estimated mortality rates between substrates conferring higher versus lower crypsis. This is similar to what we observed in **Chapter III** with the zebra finch populations: ecological population divergence, with the majority of individuals breeding where they had access to food. It is not difficult to hypothesize that such behavior should enhance individual fitness, but we were not able to corroborate it. However, in **Chapter IV**, for the first time, we successfully confirmed that the observed ecological population divergence driven by MHC was adaptive. Tree sparrows breeding in their matching area had higher reproductive success.

The second thing we experimentally corroborated for the first time is that MHC can generate positive assortative mating, as predicted (Edelaar et al., 2008; Nicolaus & Edelaar, 2018; Porter & Akcali, 2020; Porter & Benkman, 2022). In **Chapters III** and **IV** we observed how the majority of individuals bred with another individual with the same local performance as themselves, thus generating assortative mating for the new ecological trait. When individuals move to the local habitat where they have higher local performance and spend more time there, it is logical that they will meet and mate in those areas (but alternatively, mating may also

happen at a spatial scale which is larger than that of MHC or during times when MHC is not relevant). The direct consequence of this is assortative mating based on local performance. When there is one ecological trait driving MHC, as in our experiments, assortative mating for this trait is expected.. In contrast, when ecological performance depends on multiple aspects of the phenotype and the environment, assortative mating for local performance would not necessarily imply assortative mating for a specific trait. Due to the relatively small scale of the experimental setting in **Chapter III**, we observed that assortative mating was not present in extra-pair copulations. This may be because the cost of flying between areas was low, and individuals were able to easily avoid mate guarding, preventing extra-pair copulations by flying to the opposite area where their partner was feeding (Birkhead et al., 1988). However, in **Chapter IV** we observed that MHC can also promote that extra pair copulations are assortative when the spatial scale is appropriate.

Another key finding that we experimentally confirmed for the first time is that MHC can drive reproductive isolation. This is the direct consequence of MHC promoting assortative mating based on local performance. It led to reproductive isolation between the two groups of birds that differ in their ecological trait and local performance. By using parentage analysis, in **Chapter IV,** we observed reproductive isolation between the groups of birds that differ in their ecological trait and local performance. Reproductive isolation was close to complete. Thus, for the first time, we experimentally confirmed that any ecological trait involved in MHC can be considered as a 'magic trait' that promotes ecological divergence and reproductive isolation at the same time (Servedio et al., 2011).

Finally, another novel aspect of our research is that MHC can promote all the above mentioned consequences even in a new environmental setting and involving a novel ecological trait. In

both **Chapters III** and **IV,** individuals were able to adaptively select breeding areas via self-assessment of local performance based on a novel ecological trait (the transponder tag) and a novel environment (the feeders). Thus, matching habitat choice can be seen as a previously evolved mechanism that allows individuals to rapidly and adaptively respond to novel ecological challenges, as long as they are able to evaluate the effects on their local performance (Edelaar et al., 2008; Edelaar & Bolnick, 2019). This suggests that MHC could be especially relevant for rapid adaptation to novel environmental conditions, such as those associated with global change. Organisms may be able to quickly respond to these challenges as long as the new local environments affect an aspect of local performance that can be evaluated by the organisms themselves, at spatial and temporal scales that allow comparison and choice.

These findings on MHC confirm previous theoretical work and emphasize the potential impact of this mechanism on population adaptation and evolutionary dynamics. It seems that natural selection has favored the appearance of a habitat choice mechanism that, somewhat ironically, aims to prevent natural selection from acting (at least on the traits involved on MHC). When individuals move to look for a better phenotype-environment match they avoid the environment's selective pressure (Edelaar & Bolnick, 2019). This natural selection pressure can lead to a decline in phenotypic and genetic variation, and in population size. This, in turn, it can reduce the adaptive potential of a population, increase sensitivity to stochastic events, and ultimately lead to extinction (Barton & Partridge, 2000; Orr et al., 2008) (Orr et al., 2008). In contrast, MHC can protect genetic variation and reduce the demographic cost of natural selection, since pre-adapted individuals are using or colonizing different environments. This mitigates demographic costs. However, at the same time MHC can also act synergistically with natural selection. After ecological divergence emerges by MHC, it allows natural selection to

act on different traits in those different environments that would generate new evolutionary dynamics (Edelaar & Bolnick, 2012; Price et al., 2003).

Another synergistic effect could arise during ecological divergence and sympatric speciation. Sympatric speciation has traditionally been considered rare in nature, as the absence of geographical barriers can lead to gene flow that reduces the adaptive effects of divergent natural selection (Jiggins, 2006; Phillimore et al., 2008). However, as demonstrated in our study, even on a small spatial scale, MHC can drive non-random gene flow. This can promote divergence, assortative mating, and even reproductive isolation, all of which are necessary for ecological speciation to occur. It is possible that MHC first promotes or reinforces divergence, and that natural selection acting on other phenotypic traits in different local environments subsequently could lead to the evolution of additional reproductive isolation mechanisms.

So, answering the initial self-formulated question "Is MHC usually ignored because it is not relevant enough, or is it because its relevance is not yet known?", I think that we should discard the option that it is not relevant enough. Thus, we should probably focus on promoting our knowledge about it, and its integration into evolutionary ecology theory.

Next steps

We are confident that this Ph.D. thesis highlights certain directions and could serve as a catalyst for further research. Perhaps one of the most straightforward next steps would be to reanalyze/reinterpret the already published data on the heritability of phenotypic traits, taking into account the findings presented here. It would be important to determine whether the provided heritability estimates reflect the total heritability of the phenotypic trait, its heritability after controlling for environmental factors, or if the causal structure has been misinterpreted

and the estimates do not reflect any of these estimates. Being aware of this will help to understand the potential misinterpretations stated in **Chapter I**.

Another relatively straightforward step could be to analyze existing datasets from the perspective of habitat heritability as an integral part of the organism and the effect of environmental plasticity. Long-term studies with individual environmental and phenotypic data could be used for this purpose. As done in **Chapter II**, using the animal model approach and fitting the environment as a dependent variable, heritability estimates of phenotypic traits in different local environments could be estimated. Adding a phenotypic trait as a covariate should yield similar results for environmental plasticity estimates and allow their study.

If the available datasets including environmental data that individuals experience are scarce, the work done here should also stimulate the improvement of data collection, with a focus on environmental data. With the improvement and affordability of tracking devices, generating this type of datasets should become easier and could provide information about the heritability of multiple habitats that organisms interact with (e.g., Gervais et al., 2020 and 2022). Just as we currently have reviews on mean heritability estimates of different types of phenotypic traits (Postma, 2014; Stirling et al., 2002) ideally in a few years, we would have access to reviews on estimates of the heritability of the environment. This would help to clarify how the environment that individuals experience can influence their phenotypic traits and the adaptive potential of populations beyond estimates on genetic heritability.

On the other hand, I think that my Ph.D. thesis should also stimulate research on MHC prevalence and relevance in nature due to its ecological and evolutionary consequences as shown here. In this case, the animal model approach may be the easiest way to start testing this

hypothesis. By fitting a phenotypic trait as a covariate of a specific local environment, we should be able to estimate the strength of the effect of the phenotypic trait on the selection of the local environment. However, it is important to note that without complementary experimental tests, assuming a causal relationship between environment and phenotype may be speculative. As shown in **Chapter I**, this could lead to misinterpretation of the estimates.

Another challenge in testing the relevance of MHC in nature is that it may not leave easily trackable "traces" in populations. This makes it difficult to determine if MHC has generated or contributed in the past to the currently observed patterns of population divergence. Consequently, the only viable solution may be to investigate if MHC is currently influencing different populations. Based on this, and if we have enough knowledge about its occurrence in different species, we could extrapolate to identify circumstances under which MHC is likely to be more relevant. As mentioned earlier, MHC could be more prevalent in nature than previously thought and could be especially important during rapid environmental changes, such as due to global change. However, MHC could also have constraints. First, environmental changes should occur at a spatial scale that allows individuals to find different habitats. Then, individuals have to be able to assess their local performance in these habitats, and dispersal trade-offs should be positive for MHC to emerge. On the other hand, global change could also constrain MHC due to its homogenizing effect, as the lack of diverse habitats to choose from could limit its impact.

Manipulating the phenotype to induce a change in habitat preference provides strong support for the role of MHC (Edelaar et al., 2008, Camacho et al. 2020). This manipulation allows us to disentangle any previous associations with habitat preference that may be influenced by genetics or imprinting. For example, translocation experiments can help us rule out imprinting

if the translocated individuals do not return to their original habitat. However, if translocated individuals still prefer their natal habitat, it may not be possible to differentiate between direct genetic habitat choice or matching habitat choice. Despite these, manipulating the phenotype in nature can be difficult and may not always be feasible. In such cases, alternative approaches, accompanied by literature support, can be used to rule out other mechanisms, and may be useful for giving new insights into the relevance of MHC in nature (Camacho & Hendry, 2020).

Overall, we believe that this research, which emphasizes the importance of choosing or adjusting the environment to enhance fitness, will open up new avenues of research and provide insights into the complex interplay between genes, environment, and evolution in shaping phenotypic traits, environments, and population dynamics. Hopefully, this will help to improve biodiversity management and mitigate the effect of global change.

General conclusions

General conclusions

1. The local environment can be treated as an extended phenotype from a quantitative genetic perspective. Animal models can be used to estimate the heritability of the environment by fitting it as a dependent variable.
2. Animal models can also be used to estimate the strength of environmental plasticity, which is the effect of the phenotype on the environment. They serve as a tool to investigate the effects of phenotypic traits on the chosen or adjusted environment.
3. Not considering the heritability of the extended phenotype and the existence of environmental plasticity in the study system may result in model misrepresentation and estimate bias.
4. Matching habitat choice can drive population ecological divergence within a single generation, at relatively small spatial scales. This can occur even without the action of natural selection on the trait that diverges.
5. The population ecological divergence can be adaptive. Mean population fitness can increase without differential mortality driven by natural selection.
6. Matching habitat choice can indirectly lead to assortative mating and reproductive isolation. Thus, any trait affecting local performance and matching habitat choice can be considered a 'magic trait'.
7. Matching habitat choice can act based on novel traits and environments, highlighting its potential relevance in rapid environmental changes induced by global change

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Supplementary material

Chapter I

Supplementary material I: About considering the extended phenotype as a focal trait

In some simulated scenarios the focal environment is chosen or adjusted by the individual (cf. Edelaar & Bolnick, 2019). This means that the environmental variable that the individual experiences can be seen as an 'expressed trait', due to certain preferences or capabilities for choice or adjustment. Dawkins (1982) described this as the extended phenotype, as a "part of the phenotypic expression of genes" and hypothesized about geneticists studying termite mounds (an example of an extended phenotype) as a phenotypic trait and how their shape would have evolved under the effect of natural selection. Other authors have also referred to the extended phenotype as a trait expressed in the external environment (e.g., Bailey 2012) and have indeed studied the genetic underpinning of extended phenotypes (e.g., Weber et al., 2013; Järvinen et al. 2017; Gaither et al., 2018). In contrast, one could argue that an extended phenotype is only an environmental by-product of the relevant phenotypic trait expressed in the organism, e.g., the ability to build a termite mound. It would follow that the phenotypic trait that is affecting the choice or adjustment of the environment is the actual trait that has the genetic basis and is evolving, not the extended phenotype. One may therefore decide that instead of focussing on the extended phenotype it would be more reasonable to focus on the phenotypic and genetic variation of the phenotypic trait involved in the choice or adjustment of the environment. However, in some cases, it may not be possible to identify a phenotypic trait underpinning the adjustment or choice of the environment, or maybe several phenotypic traits are implicated. Additionally, the same logic of focussing on underlying traits could be

applied to the regular phenotypic traits that are often the subject of quantitative genetics research. For example, take body mass of birds. Body mass is an expressed phenotypic trait of the organism. Its expression may depend (amongst others) on growth hormone concentration (Harvey, 2013) and feeding territory size (Carpenter et al., 1983) which in turn could depend on other phenotypic traits, all with their genetic variance. Nonetheless, researchers normally do not consider body mass to be a by-product of those underlying phenotypic traits, and often do focus on the expressed body mass (e.g., Lessels & Ovenden, 1989). Related to this, there is widespread agreement that natural selection may act directly on body mass. Of course, researchers could be interested in also estimating the heritability of growth hormone concentration and taking this as the focal trait, or could be interested in disentangling the genetic effect of hormone concentration on body mass by fitting a model to body mass with hormone concentration as a covariate (de Villemereuil et al., 2018). We feel the same is true when trying to disentangle the genetic variation underpinning an extended phenotype. Hence, the expression of phenotypic traits and extended phenotypes can both be considered to be the sum of other underlying phenotypic traits, and can therefore be considered equivalents when estimating genetic variance and can be studied with the same statistical analysis. A researcher is free to choose any trait as the trait of interest. However, when investigating the heritability of the environment as an extended phenotype, the appropriate focal trait is the extended phenotype itself, i.e., the environment.

Supplementary material II: Supplementary Results

Summary table

		Model 1 ($\mathrm{E}[\hat{V}_{a_z}]$)	**Model 2** ($\mathrm{E}[\hat{V}_{a_z}]$)	**Model 3** ($\mathrm{E}[\hat{V}_{a_x}]$)	**Model 4** ($\mathrm{E}[\hat{V}_{a_x}]$)
Scenario	**1**	0	0	0	0
	2	0	0	0	0
	3	$\sigma^2_{a_z} = 25$	$\sigma^2_{a_z} = 25$	0	0
	4	$\sigma^2_{a_z} = 25$	$\sigma^2_{a_z} = 25$	0	$\beta^2_{xz}(\sigma^2_{a_z}\,\rho^2)$ = 1.87
	5	0	0	$\sigma^2_{a_x} = 12.5$	$\sigma^2_{a_x} = 12.5$
	6	$\sigma^2_{a_z} = 25$	$\sigma^2_{a_z} = 25$	$\sigma^2_{a_x} = 12.5$	$\sigma^2_{a_x} = 12.5$
	7	$\beta^2_{xz}\sigma^2_{a_x}$ = 6.13	0	$\sigma^2_{a_x} = 12.5$	$\sigma^2_{a_x} + \beta^2_{xz}[\sigma^2_{a_x}(\beta^2_{xz}\rho^2 - 2\rho)]$ = 6.99
	8	$\sigma^2_{a_z} + \beta^2_{xz}\sigma^2_{a_x}$ = 31.13	$\sigma^2_{a_z} = 25$	$\sigma^2_{a_x} = 12.5$	$\sigma^2_{a_x} + \beta^2_{xz}[\sigma^2_{a_z}\,\rho^2 + \sigma^2_{a_x}(\beta^2_{xz}\rho^2 - 2\rho)]$= 10.07
	9	0	0	0	0
	10	0	$\beta^2_{zx}(\sigma^2_{a_x}\,\rho^2)$ = 3.56	$\sigma^2_{a_x} = 12.5$	$\sigma^2_{a_x} = 12.5$
	11	$\sigma^2_{a_z} = 25$	$\sigma^2_{a_z} + \beta^2_{zx}[\sigma^2_{a_z}(\beta^2_{zx}\rho^2 - 2\rho)]$ = 10.85	$\beta^2_{zx}\sigma^2_{a_z}$ = 6.25	0
	12	$\sigma^2_{a_z} = 25$	$\sigma^2_{a_z} + \beta^2_{zx}[\sigma^2_{a_x}\,\rho^2 + \sigma^2_{a_z}(\beta^2_{zx}\rho^2 - 2\rho)]$ =16.73	$\sigma^2_{a_x} + \beta^2_{zx}\sigma^2_{a_z}$ = 18.75	$\sigma^2_{a_x} = 12.5$

Table S1. Expected values for the additive genetic estimates ($\mathrm{E}[\hat{V}_{a_z}]$ and $\mathrm{E}[\hat{V}_{a_x}]$) for each model-scenario combination. For models 1 and 2 $\rho = \frac{\sigma^2_z}{\sigma^2_x+\beta^2_{zx}\sigma^2_z}$ and for models 3 and 4 $\rho = \frac{\sigma^2_x}{\sigma^2_z+\beta^2_{xz}\sigma^2_x}$

Analysing the standard scenarios with classical animal model structures

As expected, when models 1 and 2 (the classic use of animal models) were applied to standard scenarios 1-4 where a direct genetic basis for the phenotypic trait and/or phenotypic plasticity were simulated, the expected value for the estimated genetic variance is equal to the simulated direct additive genetic variance of the phenotypic trait ($E[\hat{V}_{a_z}] = \sigma^2_{a_z}$; Fig. S1, Table S2), corroborating the appropriateness of the simulation and analytical procedures.

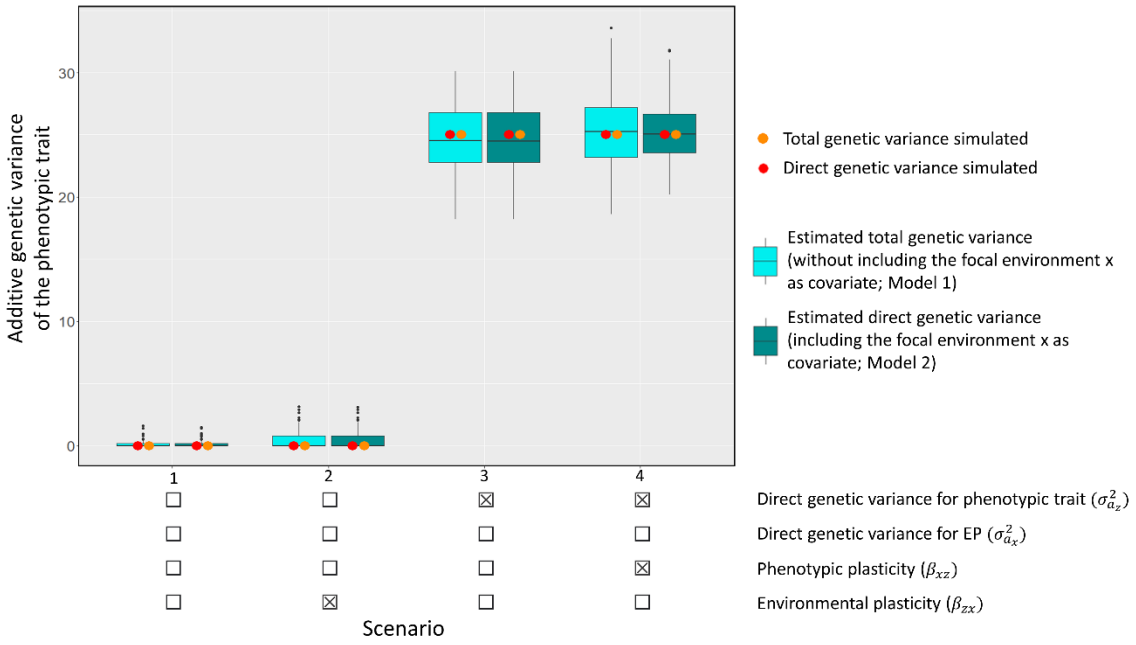


Figure S1. Additive genetic variance estimates for the phenotypic trait for models 1 (phenotypic trait as dependent variable) and 2 (phenotypic trait as the dependent variable and focal environment as a covariate) applied to the simulated data for scenarios 1 to 4. The box plots illustrate the distribution of estimates of the 100 simulations for each scenario (the bottom and the top of the boxes are the first and third quartiles, the middle band is the median, its whiskers extend from the box to highest and lowest points within 1.5 times the interquartile range. Outliers are represented with a black dot). Red dots are the simulated direct additive genetic variances for the focal phenotypic trait. Orange dots

are the simulated total additive genetic variance (direct + indirect). Crossed squares (☒) indicate if non-zero genetic variance for the phenotypic trait, genetic variance for the focal environment, phenotypic plasticity and/or environmental plasticity were simulated.

	Model 1	**Model 2**	
Scenario	$\hat{V}_{a_z}$ difference	$\hat{V}_{a_z}$ difference	$\hat{\beta}_{xz}$ differenc e
1	0.14	0.13	0
2	0.58	0.58	0
3	-0.27	-0.27	0
4	0.41	0.31	0

Table S2. Difference between the mean estimated and simulated direct value for additive genetic variance of the phenotypic trait and phenotypic plasticity with models 1 and 2 for scenarios 1, 2, 3, and 4.

What happens if the environment has a genetic basis and it is fitted as the dependent variable in an animal model?

The expected value for the estimated genetic variance is equal to the simulated direct additive genetic variance of the focal environment ($E\left[\hat{V}_{a_x}\right] = \sigma^2_{a_x}$; Fig S2, Table S3). The same was true for the estimated environmental plasticity value (Fig S3, Table S3).

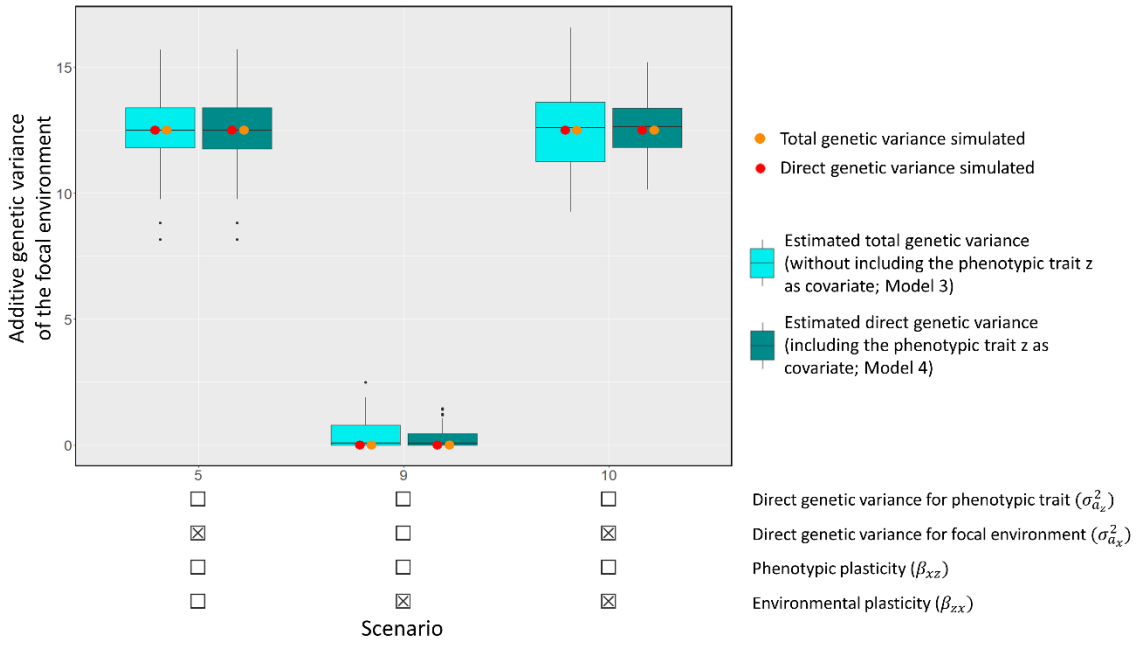


Figure S2. Additive genetic variance estimates for the focal environment by models 3 (focal environment as dependent variable) and 4 (adding the phenotypic trait as a covariate) applied to the simulated data for scenarios 5, 9, and 10. (see Fig. S1 for box plot description and legend explanation).

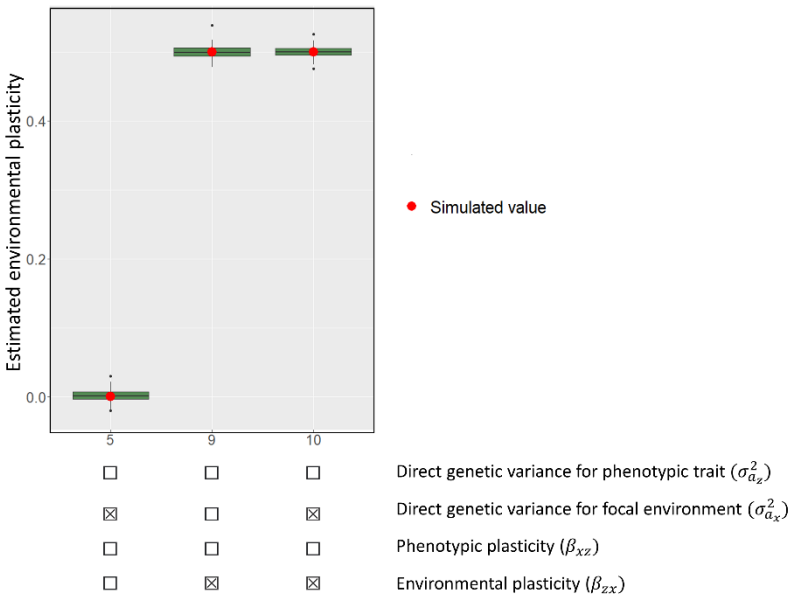


Figure S3. Estimated effects of the phenotypic trait on the focal environment b_{zx} (i.e., strength of environmental plasticity) for model 4 (phenotypic trait as a covariate) (see Fig. S1 for box plot description and legend explanation).

	Model 3	**Model 4**	
Scenari	$\hat{V}_{a_x}$	$\hat{V}_{a_x}$	$\hat{\beta}_{zx}$
o	difference	difference	difference
5	0.06	0.06	0.00
9	0.41	0.28	0.00
10	0.07	0.09	0.00

Table S3. Difference between the mean estimated and simulated direct value for additive genetic variance of the focal environment $\hat{V}_{a_x}$ and environmental plasticity β_{zx} with models 3 and 4 for scenarios 5, 9, and 10.

How are additive genetic estimates of the focal environment affected when the phenotypic trait has a genetic basis and the focal environment is affected by the phenotypic trait?

When we simulated a genetic basis for the phenotypic trait alongside a genetic basis for the focal environment, but did not simulate environmental plasticity (scenarios 3 and 6) and then applied models with the phenotypic trait as the dependent variable, without or with the focal environment as a covariate (models 3 and 4), the expected value for the estimated genetic variance is equal to the simulated direct genetic variance ($E\left[\hat{V}_{a_x}\right] = \sigma^2_{a_x}$) (Fig. S4). On the other hand, when we simulated environmental plasticity alongside a genetic basis of the phenotypic trait (scenarios 11 and 12) and applied the model with

the focal environment as dependent variable and no covariates (model 3), the total estimated additive genetic variance of our focal environment trait was higher than the direct value used for the simulation. The expected value for the estimated genetic variance is equal to the sum of the simulated direct additive genetic effects (direct genetic basis for the focal environment) and indirect additive genetic effects (direct genetic basis for the phenotypic trait proportional to the strength of environmental plasticity) ($E\left[\hat{V}_{a_x}\right] = \sigma^2_{a_x} + \beta^2_{zx}\sigma^2_{a_z}$; Fig. S4). When the phenotypic trait was added as covariate (model 3), indirect additive genetic effects were filtered out and the expected value for the estimated genetic variance is equal to the simulated direct genetic variance ($E\left[\hat{V}_{a_x}\right] = \sigma^2_{a_x}$; Fig. S4).

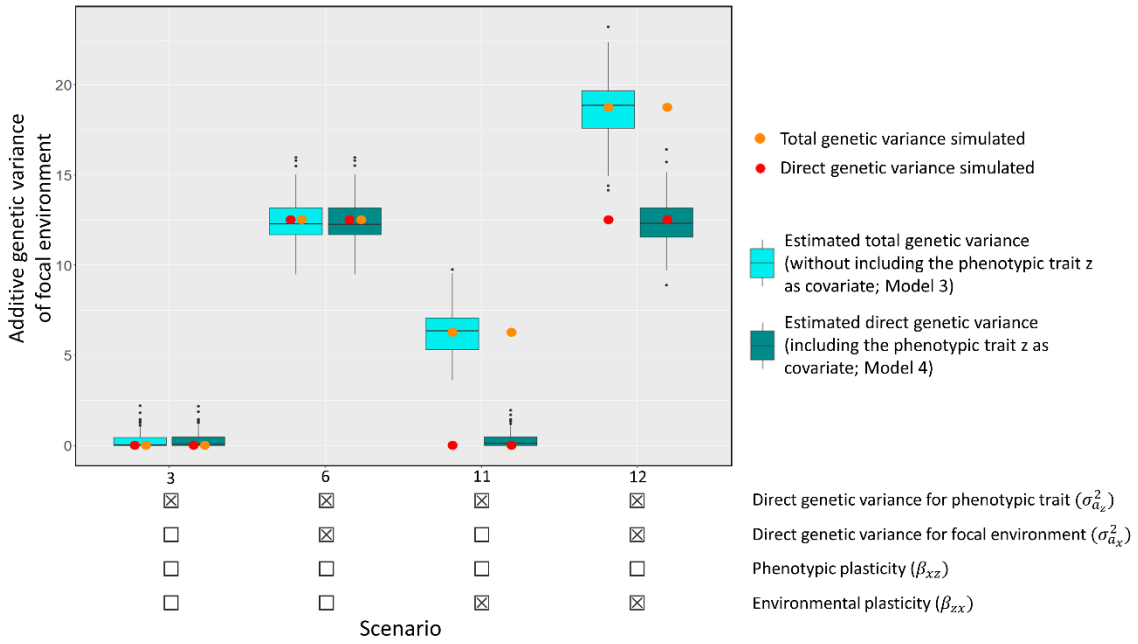


Figure S4. Additive genetic variance estimates for the focal environment for models 3 (focal environment as dependent variable) and 4 (phenotypic trait as covariate) applied to the simulated data for scenarios 3, 6, 11 and 12 (see Figure S1 for boxplot description and an explanation of the legend).

	Model 1	Model 2	
Scenari	$\hat{V}_{a_z}$	$\hat{V}_{a_z}$	$\hat{\beta}_{xz}$
o	difference	difference	difference
5	0.54	0.54	0.00
6	-0.05	-0.05	0.00
7	6.32	0.55	0.00
8	6.30	0.15	0.00

Table S4. Difference between the mean estimated and simulated direct value for additive genetic variance of the phenotypic trait and phenotypic plasticity with models 1 and 2 for scenario 5, 6, 7 and 8.

What happens if there is phenotypic plasticity yet the phenotypic trait is fitted as a covariate?

When applying the model with the focal environment as dependent variable and no phenotypic trait as covariate (model 3) to scenarios where environmental plasticity was simulated (2, 4, 7 and 8), the expected value for the estimated genetic variance is equal to the simulated direct genetic variance ($E\left[\hat{V}_{a_x}\right] = \sigma^2_{a_x}$; Fig. S5). However, when we added the phenotypic trait as covariate (model 4) and thus applied a model that assumes the wrong causal relation (assuming environmental plasticity when there is in fact phenotypic plasticity), the additive genetic variance and environmental plasticity were severely misestimated (see Discussion in the main document). Genetic variance was estimated to be present when in fact it was simulated to be absent, or it was underestimated (Fig. S5).

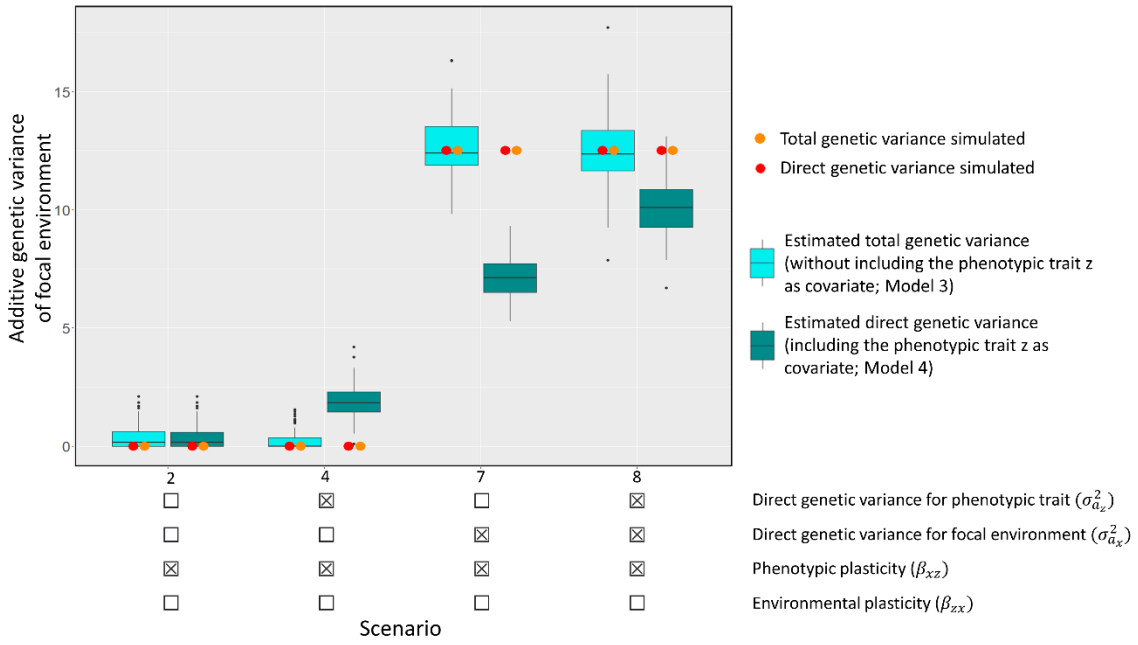


Figure S5. Additive genetic variance estimates for the focal environment for models 3 (focal environment as dependent variable) and 4 (phenotypic trait as covariate) applied to the simulated data for scenarios 2, 4, 7 and 8 (see Figure S1 for boxplot description and an explanation of the legend).

	Model 1	**Model 2**	
Scenari	$\hat{V}_{a_z}$	$\hat{V}_{a_z}$	$\hat{\beta}_{xz}$
o	difference	difference	difference
9	0.57	0.41	0.60
10	0.52	3.77	0.54
11	0.29	-13.62	0.67
12	0.05	-8.30	0.61

Table S5. Difference between the mean estimated and simulated direct value for additive genetic variance of the phenotypic trait and phenotypic plasticity with models 1 and 2 for scenario 9, 10, 11 and 12.

What if bivariate models are applied?

When applying the bivariate model (model 5) to all scenarios, estimates for the genetic variances of the phenotypic trait and the focal environment matched estimates by models 1 ($\hat{V}_{a_z}$) and 3 ($\hat{V}_{a_x}$) (i.e., univariate models without a covariate, Fig. S6).

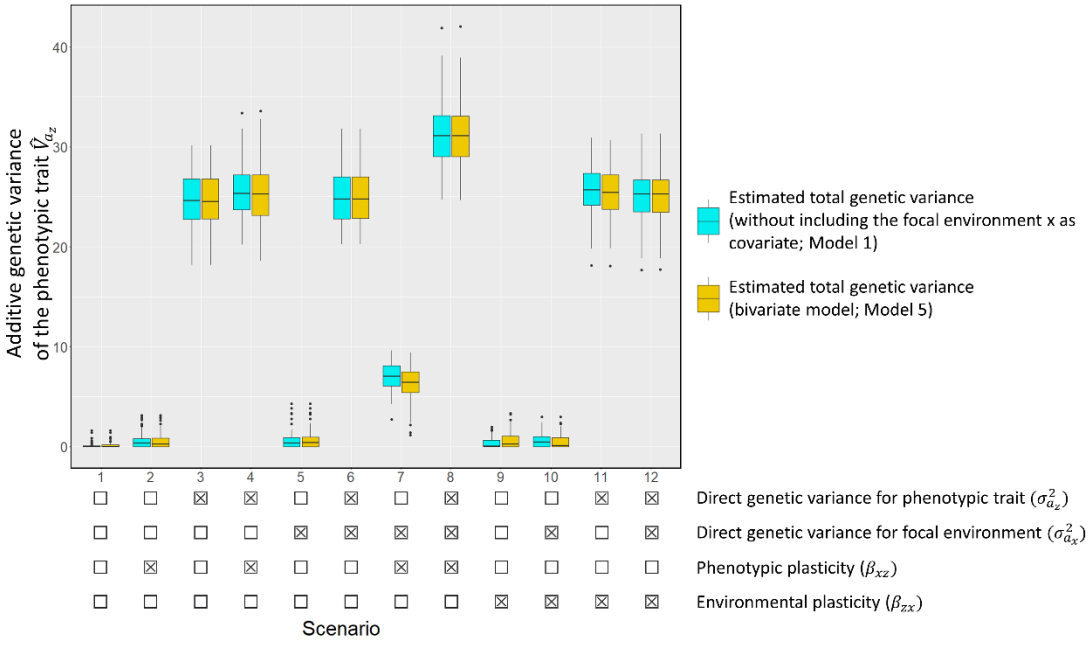


Figure S6. Distribution of the estimated values of additive genetic variance of the phenotypic trait for models 1 (phenotypic trait as dependent variable) versus model 5 (phenotypic trait and focal environment bivariate model) (see Figure S1 for boxplot description and an explanation of the legend).

Supplementary material III: Supplementary equations and mathematical approximations

Consequences of fitting the wrong model structure for estimates of additive genetic variance and strength of plasticity

Modelling the wrong causal effect has two consequences. On the one hand, the model wrongly estimates a non-zero regression coefficient for the covariate. On the other hand, it also misestimates the additive genetic variation.

In the scenario where there is environmental plasticity (β_{zx}>0) yet we structure the model assuming there is phenotypic plasticity, the expected estimate of phenotypic plasticity is a function of,

$$E[\hat{\beta}_{xz}] = \frac{\beta_{zx}(\sigma^2_{az}+\sigma^2_{ez})}{(\sigma^2_{ax}+\sigma^2_{ex}+\beta^2_{zx}(\sigma^2_{az}+\sigma^2_{ez}))}. \text{ (S2)}$$

This, in turn, affects the expected estimate of the additive genetic variance as a function of $\hat{\beta}_{xz}$,

$$E[\hat{V}_{a_z}] \approx \sigma^2_{az} + \left[\frac{\beta_{zx}(\sigma^2_{az}+\sigma^2_{ez})}{(\sigma^2_{ax}+\sigma^2_{ex}+\beta^2_{zx}(\sigma^2_{az}+\sigma^2_{ez}))}\right]^2 \sigma^2_{ax} + \left[\frac{\beta^2_{zx}\,(\sigma^2_{az}+\sigma^2_{ez})}{(\sigma^2_{ax}+\sigma^2_{ex}+\beta^2_{zx}(\sigma^2_{az}+\sigma^2_{ez}))}\right]^2 \sigma^2_{az} -$$

$$2\left[\frac{\beta^2_{zx}(\sigma^2_{az}+\sigma^2_{ez})}{(\sigma^2_{ax}+\sigma^2_{ex}+\beta^2_{zx}(\sigma^2_{az}+\sigma^2_{ez}))}\right]\sigma^2_{az} \text{ (S3)}$$

The reason for this result is that fitting the wrong causal relation generates a genetic signal in the residual values when correcting for the effects of the environment on the phenotype.

We arrived at this equation as follows. The simulation equations were,

$$z = a_z + \epsilon_z, \text{ (S4)}$$

and

$$x = \alpha_x + \epsilon_x + \beta_{zx}(\alpha + \epsilon_z). \text{ (S5)}$$

When one fits a model that assumes that x causally affects z and then estimates the conditional additive genetic variance on z, the model estimates a conditional breeding value (a_z),

$$a_z = \alpha_z - \hat{\beta}_{xz}(\alpha_x + \beta_{zx}\alpha_z) \text{ (S6)}.$$

Note that the simulated additive genetic effect is α_z and is not mean centred as opposed to the estimated breeding value (a_z). Substituting $\hat{\beta}_{xz}$with the simulated values,

$$E[a_z] \approx \alpha_z - \frac{\beta_{zx}(\sigma^2_{az}+\sigma^2_{ez})}{(\sigma^2_{ax}+\sigma^2_{ex}+\beta^2_{zx}(\sigma^2_{az}+\sigma^2_{ez}))}(\alpha_x + \beta_{zx}\alpha_z) \text{ (S7).}$$

The variance of the conditional breeding values can thus be estimated using the rules of adding variances, where the variance of the differences between two vectors is equal to the variance of the first vector a_z , plus the variance of the other vector

$$(\frac{\beta_{zx}(\sigma^2_{az}+\sigma^2_{ez})}{(\sigma^2_{ax}+\sigma^2_{ex}+\beta^2_{zx}(\sigma^2_{az}+\sigma^2_{ez}))}(a_x + \beta_{zx}a_z)) \text{ (S8),}$$

minus two times their covariance

$$Cov[az, \frac{\beta_{zx}(\sigma^2_{az}+\sigma^2_{ez})}{(\sigma^2_{ax}+\sigma^2_{ex}+\beta^2_{zx}(\sigma^2_{az}+\sigma^2_{ez}))}(\alpha_x + \beta_{zx}\alpha_z)]. \text{ (S9)}$$

The full formula the expected value for the estimated genetic variance is then,

$$\mathrm{E}[\hat{V}_{a_z}] \approx \sigma^2_{a_z} + \left(\left[\frac{\beta_{zx}(\sigma^2_{a_z}+\sigma^2_{e_z})}{(\sigma^2_{a_x}+\sigma^2_{e_x}+\beta^2_{zx}(\sigma^2_{a_z}+\sigma^2_{e_z}))}\right]^2 \sigma^2_{a_x} + \left[\frac{\beta^2_{zx}(\sigma^2_{a_z}+\sigma^2_{e_z})}{(\sigma^2_{a_x}+\sigma^2_{e_x}+\beta^2_{zx}(\sigma^2_{a_z}+\sigma^2_{e_z}))}\right]^2 \sigma^2_{a_z} - 2\left[\frac{\beta^2_{zx}(\sigma^2_{a_z}+\sigma^2_{e_z})}{(\sigma^2_{a_x}+\sigma^2_{e_x}+\beta^2_{zx}(\sigma^2_{a_z}+\sigma^2_{e_z}))}\right]\sigma^2_{a_z}\right). \text{ (S3)}$$

Where the second term of the right-hand side of the equation (everything between parentheses) captures the bias caused by fitting the wrong causal structure and is a function of the simulated additive genetic variance for both the phenotypic trait ($\sigma^2_{a_z}$) and the focal environment ($\sigma^2_{a_x}$), the residual variance of both the phenotypic trait ($\sigma^2_{e_z}$) and focal environment ($\sigma^2_{e_x}$), and the strength of environmental plasticity (β_{zx}). Equation S3 can be simplified to be represented as in the main text. If we set σ^2_z as the total direct variance of the phenotypic trait ($\sigma^2_{a_z} + \sigma^2_{e_z}$) and σ^2_x as the total direct variance of the focal environment ($\sigma^2_{a_x} + \sigma^2_{e_x}$), then we can express it as:

$$E[\hat{V}_{a_z}] \approx \sigma_{a_z}^2 + \left(\left[\frac{\beta_{zx}\sigma_z^2}{\sigma_x^2 + \beta_{zx}^2\sigma_z^2}\right]^2 \sigma_{a_x}^2 + \left[\frac{\beta_{zx}^2\sigma_z^2}{\sigma_x^2 + \beta_{zx}^2\sigma_z^2}\right]^2 \sigma_{a_z}^2 - 2\left[\frac{\beta_{zx}^2\sigma_z^2}{\sigma_x^2 + \beta_{zx}^2\sigma_z^2}\right]\sigma_{a_z}^2\right). \text{(S10)}$$

Then, we can set ρ as:

$$\frac{\sigma_z^2}{\sigma_x^2 + \beta_{zx}^2\sigma_z^2} = \rho \text{ (S11)}$$

And express S10 as:

$$E[\hat{V}_{a_z}] \approx \sigma_{a_z}^2 + \left(\beta_{zx}^2\rho^2\sigma_{a_x}^2 + \beta_{zx}^4\rho^2\sigma_{a_z}^2 - 2\beta_{zx}^2\rho\sigma_{a_z}^2\right) \text{(S12)}$$

And finally, we can collect terms for β_{zx}^2 and $\sigma_{a_z}^2$ and get Equation 11 from the main text:

$$E[\hat{V}_{a_z}] \approx \sigma_{a_z}^2 + \beta_{zx}^2[\sigma_{a_x}^2\,\rho^2 + \sigma_{a_z}^2(\beta_{zx}^2\rho^2 - 2\rho)] \text{ (11).}$$

Chapter II

	V_A (se)	V_{PE} (se)	V_Y (se)	V_R (se)	h^2 (se)	pe^2 (se)	y^2 (se)	r^2 (se)	Df
All individuals	155.51 (35.65)	196.53 (38.49)	12.35 (5.80)	467.66 (18.67)	0.19 (0.04)	0.24 (0.05)	0.01 (0.01)	0.56 (0.02)	3964
Males	281.62 (74.61)	98.85 (72.04)	6.99 (5.00)	440.72 (23.53)	0.34 (0.09)	0.12 (0.08)	0.01 (0.01)	0.53 (0.03)	1911
Females	199.29 (76.00)	125.28 (78.06)	8.74 (5.63)	505.41 (30.49)	0.24 (0.09)	0.15 (0.09)	0.01 (0.01)	0.60 (0.04)	2052
Philopatric individuals	287.49 (84.98)	0.80 (76.87)	10.80 (9.90)	450.24 (42.56)	0.38 (0.10)	0.001 (0.10)	0.01 (0.01)	0.60 (0.06)	620
Philopatric males	296.94 (67.74)	0.0009 (NA)	12.24 (14.14)	440.98 (53.76)	0.40 (0.07)	$1.2*10^{-6}$ ($9.7*10^{-8}$)	0.02 (0.02)	0.59 (0.07)	361
Philopatric females	294.58 (80.38)	0.0008 (NA)	18.72 (20.65)	432.53 (62.66)	0.39 (0.09)	$1.1*10^{-6}$ ($1.1*10^{-7}$)	0.03 (0.03)	0.58 (0.09)	258
Non-philopatric individuals	$2.6*10^{-4}$ (NA)	377.03 (26.37)	11.80 (5.86)	460.99 (20.43)	$3.1*10^{-7}$ ($8.1*10^{-9}$)	0.44 (0.03)	0.01 (0.01)	0.54 (0.03)	3343

Table S1. Results of univariate animal models of female different subset of individuals. Estimates of variance components are given with their standard error: additive genetic variance (V_A), permanent environmental variance (V_{PE}), among-study year variance (V_Y), and residual within-individual variance (V_R). Estimates of heritability (h^2), permanent environmental effects (pe^2), year variance (y^2), and residual variance (r^2). And model degrees of freedom (Df)

	V_A mean (se)	V_{PE} mean (se)	V_Y mean (se)	V_R mean (se)	h^2 mean	pe^2 mean	y^2 mean	r^2 mean	Df
Philopatric simulated	163.40 (0.60)	173.32 (0.63)	13.11 (0.05)	481.43 (0.29)	0.20	0.21	0.02	0.58	3964
Dispersers simulated	157.90 (0.47)	195.03 (0.55)	11.66 (0.03)	471.26 (0.23)	0.19	0.23	0.01	0.56	3964
Immigrants simulated	176.51 (0.57)	102.41 (0.74)	16.06 (0.12)	554.13 (0.61)	0.21	0.12	0.02	0.65	3964
All	185.35 (0.94)	81.30 (1.12)	16.16 (0.13)	568.52 (0.78)	0.22	0.10	0.02	0.68	3964
Philopatric simulated (A)	6.67 (0.40)	294.97 (0.52)	12,72 (0.05)	531.58 (0.40)	0.01	0.35	0.02	0.63	3964
Dispersers simulated (A)	154.73 (0.71)	161.34 (0.77)	11.41 (0.05)	506.35 (0.32)	0.19	0.19	0.01	0.61	3964
Immigrants simulated (A)	55.97 (0.55)	23.86 (0.68)	21.53 (0.16)	730.96 (0.66)	0.07	0.03	0.03	0.87	3964
All (A)	3.91 (0.24)	4.81 (0.30)	18.48 (0.17)	821.43 (0.62)	0.005	0.01	0.02	0.97	3964

Table S2. Mean value of estimates of variance components additive genetic variance (V_A), permanent environmental variance (V_{PE}), among-study year variance (V_Y), and residual within-individual variance (V_R) and mean value of estimates of heritability (h^2), permanent environmental effects (pe^2), year variance (y^2), and residual variance (r^2). Standard error represents the standard error of that mean value not for each estimate of each model as in Table S1. For all mean estimates of h, pe^2, y^2 and r^2 se was < 0.0001. And model degrees of freedom (Df).

Chapter III

Results

Fixed effect	Estimate ± standard error	Degrees of freedom	$\chi 2$	*p*-value
Intercept	-1.7919±1.1977	-	-	-
Male transponder type	2.4162±1.3193	1	4.0157	0.045
Female transponder type	2.0323±1.1489	1	3.3177	0.069
Experiment	0.2186±1.2863	1	0.0290	0.865

Table S1. Model estimates for matching habitat choice for breeding area for heterosexual pairs (visual observations). Binomial GLM (N=23).

Fixed effect	Estimate ± standard error	Degrees of freedom	$\chi 2$	*p*-value
Intercept	-2.1824±1.4630	-	-	-
Transponder type	2.7411±1.7846	1	24.0110	9.580×10^{-7}
Sex	0.6301±0.9741	1	2.2913	0.130
Experiment	0.5767±0.7847	1	0.6970	0.404
Transponder type:Sex	-1.0838±1.5351	1	0.51253	0.474

Table S2. Model estimates for matching habitat choice for breeding area for the entire breeding population (visual observations). Binomial GLMM (N=70). Individual ID was fitted as random with an estimated variance component of 0.74±0.86 (SD).

Fixed effect	Estimate ± standard error	Degrees of freedom	$\chi 2$	*p*-value
Intercept	0.8521±0.4937	-	-	-
Nº of matching individuals	0.1061±0.2354	1	0.2079	0.648
Experiment	-0.2536±0.2957	1	0.72326	0.395

Table S3. Model estimates for reproductive success. Poisson GLM (N=23).

Fixed effect	Estimate ± standard error	Degrees of freedom	$\chi 2$	*p*-value
Intercept	-2.359±1.476	-	-	-
Female transponder type	2.291±1.356	1	8.0714	4.497×10^{-3}
Experiment	1.034±1.071	1	1.2576	0.262

Table S4. Model estimates for matching habitat choice by genetic mothers. Binomial GLMM (N=34). Individual ID was fitted as random with an estimated variance component of 0.22±0.47 (SD).

Fixed effect	Estimate ± standard error	Degrees of freedom	$\chi 2$	*p*-value
Intercept	-1.6680±1.4630	-	-	-
Male transponder type	0.8860±0.8322	1	1.2248	0.268
Experiment	1.4730±0.9855	1	2.8984	0.089

Table S5. Model estimates for matching habitat choice by genetic fathers. Binomial GLMM (N=35). Individual ID was fitted as random with an estimated variance component of 1.21±1.10 (SD).

Fixed effect	Estimate ± standard error	Degrees of freedom	$\chi 2$	*p*-value
Intercept	-1.3162 ± 1.0464	-	-	-
Transponder type	2.7542± 1.4918	1	1.5435	0.214
Extra-pair	1.5334 ± 1.2376	1	0.0266	0.871
Experiment	0.6119 ± 0.9309	1	0.6112	0.434
Male transponder type:Extra-pair	-3.4116 ± 1.9847	1	4.3169	0.038

Table S6. Model estimates for matching habitat choice by genetic fathers including their within-pair/extra-pair condition. Binomial GLMM (N=33). Individual ID was fitted as random with an estimated variance component of 0.19±0.44 (SD).

Fixed effect	Estimate ± standard error	Degrees of freedom	$\chi 2$	*p*-value
Intercept	0.9229 ± 1.1351	-	-	-
Transponder type	1.3457 ± 0.6515	1	5.6484	0.017
Experiment	-0.6287 ± 1.2871	1	0.2417	0.623

Table S7. Model estimates for matching habitat choice for within-pair genetic fathers. Binomial GLM (N=17).

Methods

Removing individuals before the experiment

Some birds were removed from the experimental aviary before starting the experiment either because (i) they had health issues; (ii) they were not able to use the feeders; (iii) they were thought to feed on spilled seeds or "steal" seeds from feeders in the area where they were supposed to not have access to food; (iv) there was an unbalanced number of males and females. More details are provided below to facilitate future studies using a similar approach.

(i) **Birds with health issues.** We received some birds from the suppliers already in poor health. Some were unable to maintain flight for more than a few seconds (an impediment to move between breeding areas, and for breeding in general). We also observed some birds that were affected by the transponder tag. These suffered from a swollen tarsus, which was aggravated by the transponder. In some birds, the swelling was due to infections with scaly leg mites (which were subsequently treated with ivermectin, 0,12%). In others, it seems that the provided egg food or dirt (including scaly material from the tarsus) was accumulating between the transponder tag and the leg and that was causing some kind of infection. This situation was improved by providing the egg food in closed feeders so birds were not able to perch on the food. Birds in this condition were treated with an extract of *Centella asiatica* in the form of ointment (10 mg/g, Blastoestimulina) and we changed their transponder tag to their other "healthy" leg. Even so, some individuals remained with some swelling. This prevented them from carrying the transponder tag, and for this reason they were removed.

(ii) and (iii) **Birds to which the experimental treatment could not be properly applied.** Some birds were not able to learn how to use the feeders. The reason is unknown but these birds were registered at a very low frequency (compared to their conspecifics) or not registered at all at the electronic feeders. Thus, the experimental treatment, to provide access to feeders (and food) in only one breeding area, could not be applied to them and, moreover, they were at risk of starvation. Because of this they were removed. On the other hand, some birds were registered at a high frequency (compared to their conspecifics) at the electronic feeders where they were supposed not to have access to food. These birds were either unable to learn where they had access to food (because they keep trying to get seeds from the wrong feeders), or somehow they were able to feed from spilled seeds on those feeders. We improved the latter by placing grids under the feeders to collect the seeds that fell from the feeders when a bird was feeding and thereby prevent other birds from having access to those seeds. In either case, the experimental treatment (giving access to food in only one breeding area) was not successful for those birds, so they were removed.

(iv) **Birds removed to balance the female:male ratio.** In experiment 1, the acclimation phase started with the same number of males and females. After removing some individuals due to points i, ii and iii, 3 additional males were removed to reach a 1:1 sex ratio. In experiment 2, the acclimation phase started with an unequal number of males and females. After removing some individuals due to points i, ii and iii above, 19 additional males were removed. We entered 32 females and 38 males in the experiment. We maintained 6 “spare” males due to the relatively high proportion of female same-sex pairs that bred in experiment 1, to make sure that this wasn't due to the lack of suitable male partners.

Genotyping

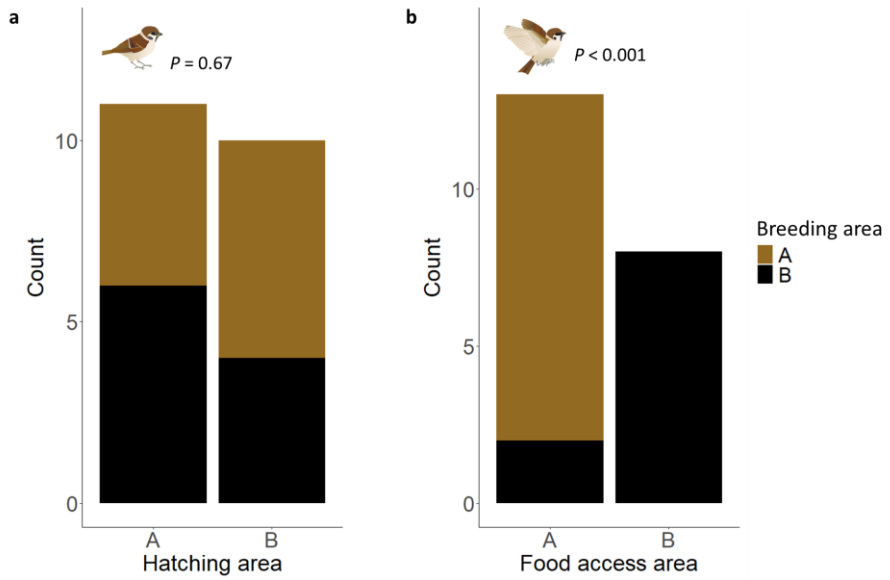
chromosome	primer name	fluorescence label	mix	mix volume	volume of primer (stock concentration 100µM)	annealing temperature	N of cycles
Tgu1A	chr1A_39MB	NED	1	300	0.7	60°C	23
Tgu3	chr3_58MB	PET	1	300	1.3	60°C	23
Tgu5	chr5_34MB	PET	3	200	0.8	59°C	30
Tgu6	chr6_16MB	VIC	3	200	6	59°C	30
Tgu11	chr11_8MB	NED	3	200	0.5	59°C	30
Tgu14	chr14_9MB	NED	2	200	0.5	60°C	25
Tgu15	chr15_6MB	6FAM	1	300	1.5	60°C	23
Tgu22	chr22_3MB	VIC	1	300	0.7	60°C	23
Tgu26	chr26_3MB	6FAM	2	200	1	60°C	25
Tgu27	chr27_1MB	6FAM	3	200	0.5	59°C	30

Table S8. Primers and PCR conditions for the 10 microsatellite markers used for parentage assignment.

Chapter IV

Results

To investigate the potential alternative explanation that the observed ecological population divergence could be due to the effects of imprinting, we analyzed another subset of individuals. In these analyses, we focused on individuals with a successful application of the experimental treatment and that were ringed as nestlings in either area A or B in previous years, prior to the experiment (n=21). To test for an effect of imprinting, we used Fisher's exact test to compare the relationship between the area where they were born and where they bred. Additionally, for the same subset of individuals, we used Fisher's exact test to determine if there was an association between their breeding area and their transponder group/local performance to test for matching habitat choice as before. We found no relationship between the area where they were born and the area where they bred (Extended Data Fig 1a), yet the effect of local performance on breeding area was maintained (Extended Data Fig 1b).



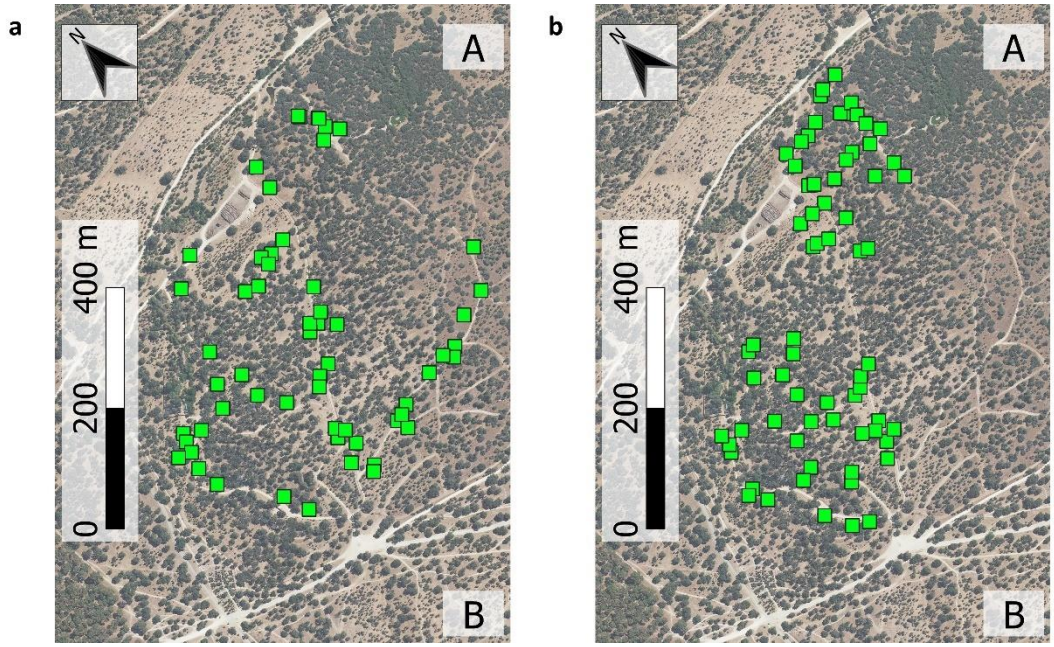
Extended Data Figure 1. Ecological population divergence was not driven by habitat imprinting and divergent natural selection. For individuals born within the study area, a, the choice of breeding area did not depend on where an individual was born (Fisher's exact test, P = 0.67). b, In contrast, their breeding area did depend on local ecological performance (Fisher's exact test, P < 0.001).

Tables

Fixed effect	Estimate ± standard error	$\chi 2$	Degrees of freedom	*p*-value
Intercept	16.01±0.62	-	-	-
Number of matching individuals per pair	0.64±0.73	0.057	1	0.811

Extended Data Table 1, GLMM assessing the effect of number of matching parents per pair on nestling weight. N=126. Brood ID was fitter as random with an estimated variance component of 2.8217.

Methods



Extended Data Figure 2. Location of nest boxes (green squares) in the study area. **a,** before the experiment and **b,** during the experiment when were placed by grouping them in area A and area B.

Supplementary video

Available at Zenodo repository

Munar-Delgado, Gabriel, Pulido, Francisco, & Edelaar, Pim. (2023). Experimental evidence for performance-dependent movement as an alternative driver of adaptive divergence. Zenodo. https://doi.org/10.5281/zenodo.7844898

	Locus	Final primer concentration (µM)	Fluorescent dye	Reference
Multiplex I	Pdoµ3	0.5	HEX	(Griffith et al., 1999)
	Ctc105	0.5	HEX	(Tarvin, 2006)
	ZC02	0.5	6-FAM	(Tarvin, 2006)
	Pamo12	0.5	TAMRA	(Izumi et al., 2009)
	Pamo13	0.5	ROX	(Izumi et al., 2009)
Multiplex II	Pamo1	0.8	TAMRA	(Izumi et al., 2009)
	Pamo3	0.3	ROX	(Izumi et al., 2009)
	Pamo7	0.3	6-FAM	(Izumi et al., 2009)
	Pdoµ5	0.3	HEX	(Griffith et al., 1999)

Extended Data Table 2. Microsatellite loci information