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

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Ecological divergence without natural selection

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

Abstract

Adaptive divergence has long been a core topic in the field of evolutionary biology, with natural selection traditionally considered its only driver. Here we focus on the ability of matching habitat choice to generate population divergence and reproductive isolation. This alternative mechanism of divergence considers that individuals choose their habitats based on an evaluation of the ecological match between their phenotype and the available environments, which subsequently limits gene flow. To test this, we conducted experiments with captive zebra fiches equipped with transponder-tags and using transponder-operated bird feeders. We thereby created within a single aviary two areas with distinct resources, and the associated ecological traits that provided access to only one of the resources. We found that most zebra finches chose to breed in the same area as where they had access to their ecological resource, thereby creating population divergence in the absence of current natural selection on the ecological trait. This choice of breeding area indirectly resulted in assortative mating for the ecological trait. If the ecological trait were heritable, this assortative mating would carry the obtained divergence into the next generation. Our results experimentally confirm the predicted ability of matching habitat choice to drive rapid population divergence and limit maladaptive gene flow, especially at the small temporal and spatial scales where natural selection is unlikely to do so (here: one generation and one aviary). This might be increasingly relevant in a world where anthropogenic impacts create rapid environmental changes.

Lay summary

Species face unprecedented environmental challenges due to human activities rapidly altering habitats and biological communities. Understanding how species adapt is therefore crucial for conservation of endangered species and management of invasive species. Natural selection, the cornerstone of adaptive evolution, has long been considered the only driver of adaptation to the environment. Our study delves into an intriguing alternative driver called Matching Habitat Choice (MHC), where animals select their environments based on their evaluation of how well their traits match ecologically with the available local environments. Using captive zebra finches in an aviary, our research examined how these birds make choices about where to settle and breed in relation to locally available food resources. We observed that these birds

preferred breeding sites matching their ecological traits. We therefore confirmed that population divergence is possible without current natural selection. Our findings carry significant implications for biodiversity management amidst rapid environmental changes. They underscore the power of individual decision-making in shaping evolutionary dynamics, offering insights for ways to preserve species in an ever-changing world.

Introduction

Immersed in the Anthropocene, species are experiencing a period of unprecedented novel and rapid environmental changes (Waters et al., 2016) leading 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; 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). MHC is thereby responsive to an individual's ecological performance and therefore to variation in phenotypes. By doing so, MHC has been hypothesized to influence a large number of ecological and evolutionary phenomena, such as individual and population fitness, local adaptation, maintenance of genetic variation, ecological population divergence, 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).

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 interpretations (Edelaar et al., 2019; Edelaar & Bolnick, 2012). 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 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 maintains genetic variation at the metapopulation level, translates into reduced gene flow between individuals with different phenotypes, 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 (here: individual food intake rate) across local environments. We created a spatially-structured environment by placing two sets of transponder-operated feeders at the two extremes of an aviary. We manipulated individual variation in local performance 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 to have access 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. We determine where each individual bred and with whom through behavioural 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 thereby 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.

Materials and Methods

Experimental design

We performed two experiments. In experiment 1 we used 70 zebra finches obtained from different providers. In experiment 2, we used 130 zebra finches raised in our experimental aviary (offspring of individuals different from those in experiment 1). Birds were uniquely colour-banded and equipped with a leg-band with a passive integrated transponder (PIT)-tag (Eccel Technology Ltd.) for visual and electronic identification.

The experimental indoor aviary was divided into four interconnected areas delimited by plastic mesh (Fig. 1). Several mosquito net strips were placed in the two intermediate areas to increase the difficulty of flying between the extreme areas. These extreme areas, designated as breeding areas A and B, were equipped with perches and

water stations throughout the entire period, encompassing both acclimation and experimental phases. Additionally, feeders and nest boxes were positioned within the breeding areas during the experiment (Fig. 1).

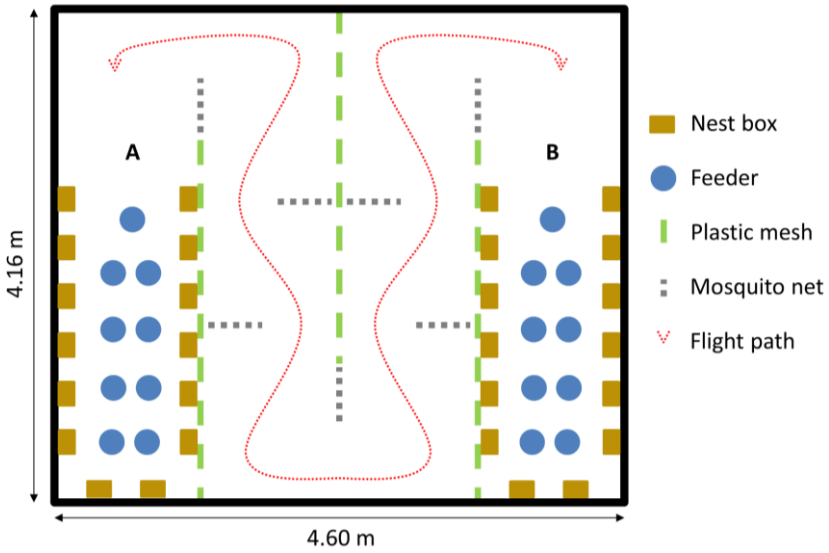

Figure 1. Schematic representation of the experimental aviary. A and B denote the name of each breeding area.

We used 18 electronically operated feeders (NatureCounters) able to read and respond to PIT-tags to manipulate individuals' local ecological performance. These feeders where usually closed but gave access to seeds when a specific birds with a specific PIT-tag perched on them.

After the acclimatization phase (Supplementary material), we retained 28 males and 28 females for experiment 1 and 38 males and 32 females for experiment 2 (Supplementary material). Subsequently, for both experiments, we placed half of the feeders in each breeding area (A and B). Following this, we randomly assigned half of the individuals to group A, providing them access to seeds only at feeders in area A, and the other half to group B, allowing them access to seeds only in area B. This method generated two distinct groups with differing ecological performances (food access) in both breeding areas. Next, we placed nest boxes in both breeding areas (28 in experiment 1 and 34 in experiment 2, half of them in each area) and let the individuals breed.

Nests were checked weekly, starting three weeks after placing the nest boxes, continuing until the last fledgling left its nest. Nestlings were banded at the age of 10±3

days (value ± range). Breeder identification was based on colour bands, direct visual observations, and camera recordings (network video recorder and 1080P camera, Sannce) (Supplementary material). 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 eggs laid after the first fledgling had left its nest (i.e., removed potential second clutches).

The experiments were performed in accredited installations (ES410910008004) and approved by the relevant authorities (Consejería de Agricultura, Pesca y Desarrollo Rural of the Junta de Andalucía, 28/03/2018/040).

Genotyping and parentage analysis

Blood samples were obtained from all adults and fledglings at the end of the experiments. DNA isolation was performed using an extraction robot (Freedom EVO 100, Tecan), and followed by genotyping at 10 microsatellite loci (Forstmeier, Schielzeth, et al., 2007, Table S9) using PCR (see Supplementary material for PCR conditions).

For parentage analysis, we utilized CERVUS version 3.0.7 (Marshall et al., 1998), employing a likelihood-based approach to determine parentage. Using specific simulation settings for each experiment (10,000 offspring, all adult individuals as candidate parents, 0.01 rate of loci mistyped), we assigned parents based on the highest log-likelihood ratio score (LOD) without specifying known parents. This allowed us to identify genetic parents of fledglings and associate each fledgling with two reproductive adults, linking each parent to the nest(s) and area(s) where its fledglings hatched.

Establishment of phenotype-environment matching

Breeding individuals (see criteria in Supplementary material) 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.

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. Consequently, at the genetic level, reproductive individuals could have been 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-matching. For this we assumed that offspring inherited the transponder type from their genetic parents in the following way. If both genetic parents had the same transponder type, the offspring inherited the transponder type of their parents. However, if genetic parents had different transponders, each offspring was assigned to inherit either transponder type with an equal probability. Finally, we compared the virtually 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, 2020). 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 MHC using a binomial GLM with breeding area as the binary response variable, and transponder type (A/B) for both breeding males and females as the explanatory variable. 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; see Supplementary material). To simplify the model, we excluded two pairs with a repeated (polygynous) male. To increase sample size, we also tested for MHC for the whole breeding population, including 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 (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 MHC for breeding had an effect on reproductive success (i.e., led to adaptation). For this, we tested whether the number of matching adults per breeding pair (0, 1 or 2) affected their reproductive success (GLM, with the number of 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 factor was 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 a focal nest due to an extra-pair fertilization) and its interaction with male transponder type as a fixed effect. To classify genetic males (fathers) as either within-pair or extra-pair mates, 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 in the opposite area).

For the models above, we only included unique individual-nest combinations for the same reasons as for testing MHC for breeding individuals.

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

After identifying extra-pair paternity (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 observed proportion of male-female genetic combinations that were assortative for transponder type differed from that expected if extra-pair mating was 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 observed 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

As is usual for studies on zebra finches, not all individuals established pair bonds (Griffith et al., 1999). In addition, some individuals established pair bonds with an individual of the same sex, or with two individuals (Tables S1 and S2).

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. 2A; Table S3). 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. 2B; Table S4). The interaction effect between transponder type and sex was not significant (LRT: $\chi 2$ (1) = 0.513, p = 0.474).

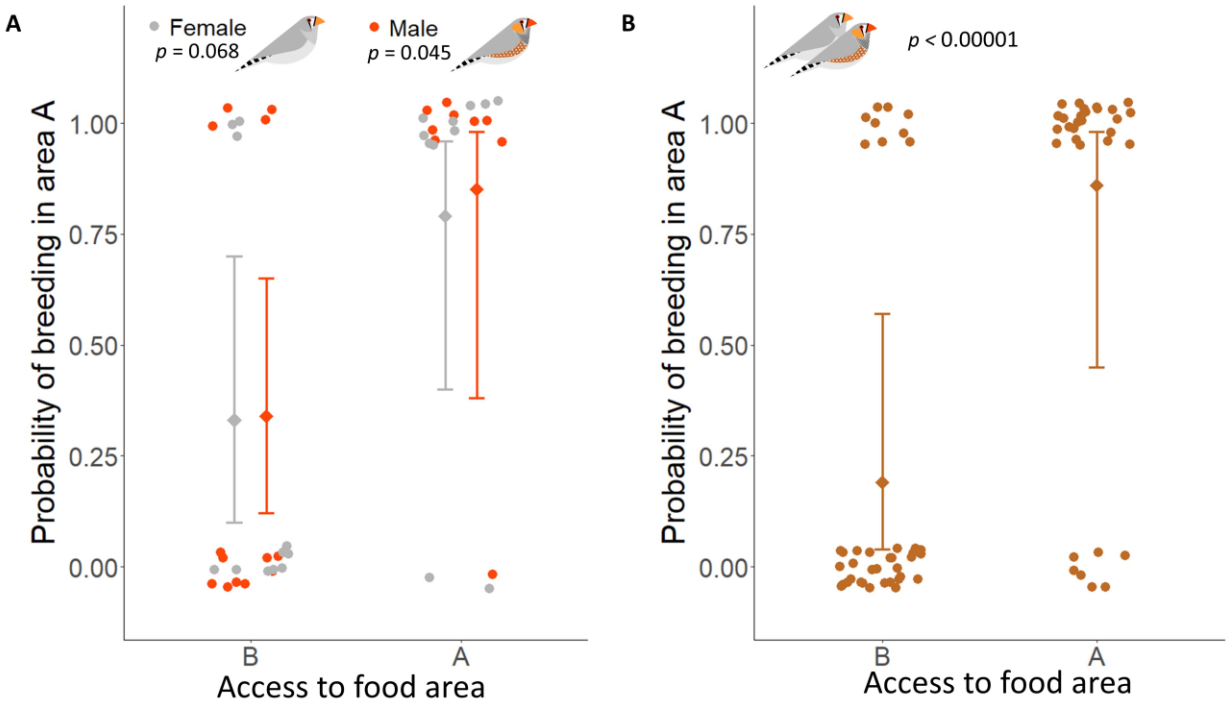

Figure 2. Matching breeding habitat choice as based on nest location. (**A**) Model predictions (binomial GLM) for the probability of breeding in area A for females (grey diamonds) and males (red diamonds) depending on their transponder type, where remaining covariates are set to their means. Error bars represent the 95% CI of model predictions. Dots are raw data for breeding females (grey) 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. 3).

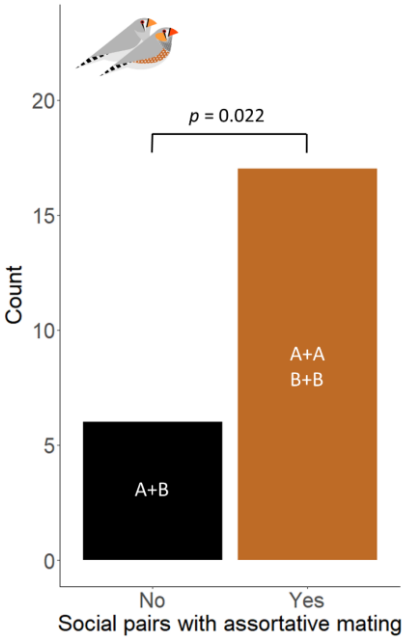

Figure 3. Assortative mating for transponder type with respect to the social mate. Number of observed heterosexual disassortative or assortative breeding pairs (letters indicate the transponder type of the two pair members).

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. 4; Table S5).

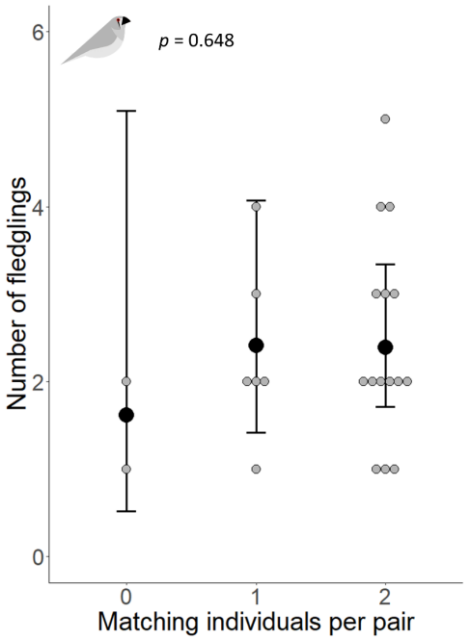

Figure 4. Reproductive success for breeding pairs. Number of fledglings for pairs with 0, 1 or 2 individuals whose transponder matches the breeding area. Black dots represent the model estimates and the error bars their 95% CI. Gray dots represent raw data.

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. 5A; Table S6). 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. 5A; Table S7). We tested if this smaller difference in males was because extra-pair offspring were predominantly produced in the area where they did not nest. 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. 5B; Table S8). 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), at 0.39 [0.09, 0.81] versus 0.55 [0.11, 0.93] respectively.

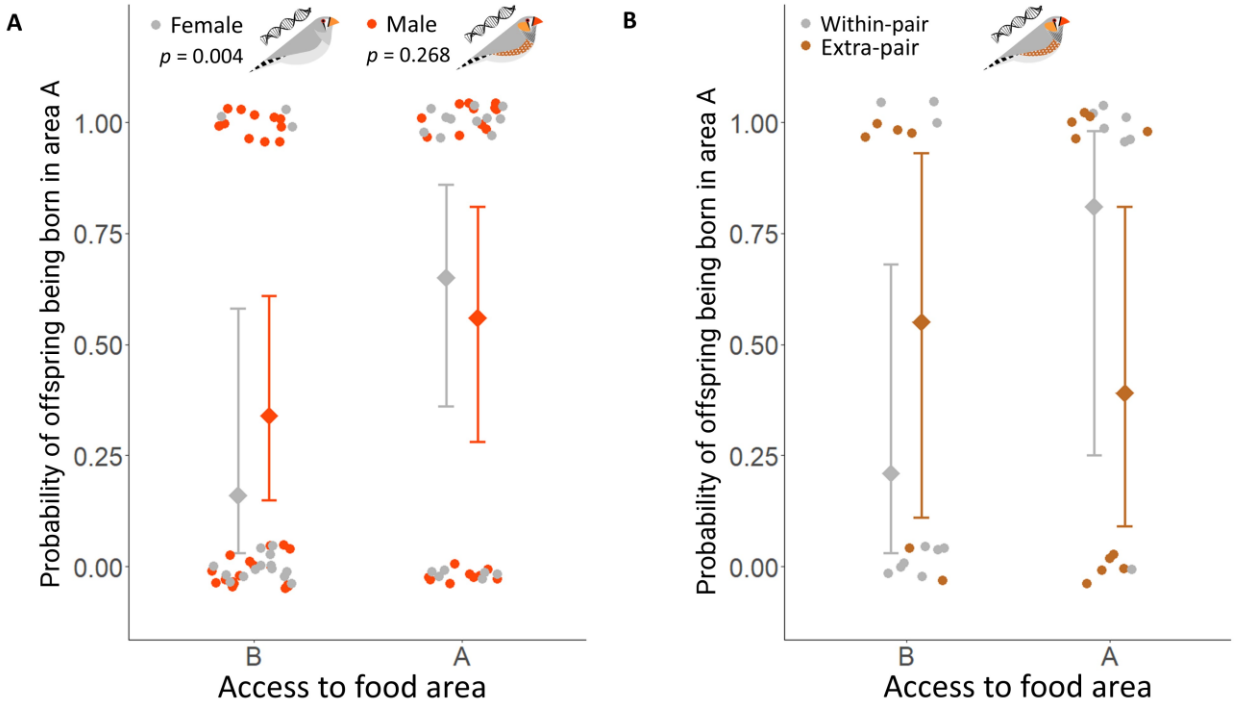

Figure 5. **Matching breeding habitat choice at the genetic level.** (**A**) Model predictions (binomial GLMM) for the probability of having genetic offspring in area A for females (grey diamonds) and males (red diamonds) depending on their transponder type. Remaining covariates are set to their means. Error bars represent the 95% CI of model predictions. Dots are raw data for females (grey) and males (red). (**B**) Model predictions (binomial GLMM) for the probability of having genetic offspring in area A for within-pair males (grey 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 (grey) 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 ($\chi2$ (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 ($\chi2$ (1) = 1.470, p = 0.225).

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

The number of locally matching fledglings (assuming virtual inheritance of parental transponders; see methods) was higher than expected by chance (53 out of 80, 66%) ($\chi2$ (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%; $\chi2$ (1) = 25.97, p < 0.000001).

Discussion

Our study provides the first demonstration, to our knowledge, that 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; Wadgymar et al., 2022).

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 kilometres 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 kilometres due to very high dispersal rates (Forstmeier, Segelbacher, et al., 2007; 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 apparently because they did not *want* to fly more. In the wild, the trade-off between flight distance and food intake will often favour 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 experimental results are largely 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; Camacho & Hendry, 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, 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 unlikely 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 aspects 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 et al., 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; Sultan et al., 2022).

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, as predicted by other studies (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 behaviour (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 mating 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. Hence, its within-generation ecological consequences become between-generation evolutionary effects. In the hypothetical extreme case that individuals responded so strongly to the habitat variation that they all would only have 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. In effect then, 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. Indeed, in a similar experiment performed in the wild, we observed such a very high degree of reproductive isolation (Munar-Delgado et al., unpublished ms).

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 circumstances.

Data and code availability

Data and scripts used for analysis are available in the Zenodo repository doi.org/10.5281/zenodo.10039728 and doi.org/10.5281/zenodo.10039712

Author contributions

Conceptualization: GMD, JLT, JP, WF, PE; Methodology: GMD, JLT, JP, WF, PE; Investigation: GMD, PHR, GSM, PE; Software: GMD; Data curation: GMD; Formal analysis: GMD, PE; Visualization: GMD; Funding acquisition: PE, JLT, JP; Resources: PE, GSM; Project administration: PE; Supervision: PE; Writing – original draft: GMD, PE; Writing – review & editing: GMD, PHR, GSM, JP, JLT, WF, PE.

Funding

Spanish Ministry of Universities grant FPU17/06268 (GMD), Spanish Ministry of Science and Innovation PID2019-108971GB-I00 (PE), Spanish Ministry of Economy & Competitiveness grant CGL2013-49460-EXP (PE).

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

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Supplementary material

Supplementary methods

Housing and acclimatation phase

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 colour phenotypes (fully white individuals were avoided since they were hard to sex). Upon arrival in the lab, birds were uniquely colour-banded 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 bred by ourselves and were the offspring of adults obtained from the same providers as for experiment 1. They 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.

Adult male and female birds from experiment 1 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 centres 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 below). 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.

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 below). 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.

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.

Identification of breeders

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 colour band combination of the individuals entering or leaving the nest box. We then associated breeding individuals with a nest based on individuals' behaviour 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 behaviour 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 behaviour. 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).

PCR conditions

DNA was isolated using an extraction robot (Freedom EVO 100, Tecan). All DNA samples were genotyped at 10 microsatellite loci (Forstmeier et al., 2007, Table S9) 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 H2O and 1 µl of DNA (Table S9). 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.

Results

Data summary

Table S1. **Overview of where zebra finches bred, and with whom**. 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

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 S2. Overview of where female or male zebra finches produced offspring. Based on genetic parentage analysis. See Methods for a description of each classification.

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

Table S3. 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	-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 S4. 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	-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 S5. Model estimates for reproductive success. Poisson GLM (N=23).

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 S6. 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	-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 S7. 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	-1.6680±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 S8. 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	-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

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 S9. Primers and PCR conditions for the 10 microsatellite markers used for parentage assignment.

Supplementary references

Forstmeier, W., Schielzeth, H., Schneider, M., & Kempenaers, B. (2007). Development of polymorphic microsatellite markers for the zebra finch (Taeniopygia guttata). *Molecular Ecology Notes*, *7*(6), 1026–1028. https://doi.org/10.1111/j.1471-8286.2007.01762.x

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