

Pairing dynamics and the origin of species

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Whether sexual selection alone can drive the evolution of assortative mating in the presence of gene flow is a long-standing question in evolutionary biology. Here, we report a role for pairing dynamics of individuals when mate choice is mutual, which is sufficient for the evolution of assortative mating by sexual selection alone in the presence of gene flow. Through behavioural observation, individual-based simulation and population genetic analysis, we evaluate the pairing dynamics of coral reef fish in the genus *Hypoplectrus* (Serranidae), and the role these dynamics can play for the evolution of assortative mating. When mate choice is mutual and the stability of mating pairs is critical for reproductive success, the evolution of assortative mating in the presence of gene flow is not only possible, but is also a robust evolutionary outcome.

Keywords: speciation; sexual selection; assortative mating; marine; *Hypoplectrus*; matching

1. INTRODUCTION

The evolution of assortative mating constitutes a key component of the speciation process [1–10]. This is particularly relevant in sympatry or parapatry, where members of diverging species may have direct courtship and mating interactions. In this context, theory predicts that the evolution of assortative mating is characterized by weak selection for increased assortment unless there is strong disruptive natural selection and the costs of assortative mating are low [2,5–8,11]. A principal cost of assortative mating results from the reduced number of mating encounters experienced by rare morphs, thus constraining the evolution of assortative mating in models of speciation with gene flow [2,5,7,8,11–16]. Yet, assortative mating at the species level is so strong and ubiquitous in nature that our ignorance of its place during the evolutionary sequence between interbreeding populations and reproductively isolated species has been referred to as the ‘missing link’ in evolution [17].

Hamlets (*Hypoplectrus* spp., Serranidae, inset in figure 1) represent a promising opportunity to study the evolution of assortative mating. This genus of Caribbean coral reef fishes is characterized by spectacularly different colour morphs, up to nine of which may be found on a single reef (figure 1), and most of which are recognized as named species. To date, no ecological or morphological trait other than colour pattern has been shown to clearly distinguish the different hamlet morphs [18–20]. Hamlets are simultaneous hermaphrodites that engage in an elaborate courtship and spawn in pairs, alternating sex roles, typically mating on a daily basis [21]. Different colour morphs are seen spawning in close proximity, yet mating is strongly assortative with respect to colour pattern, with more than 96 per cent of pairings occurring between members of the same colour morph [18,22,23]. We have previously proposed [23] that assortative mating in the hamlets is most simply explained by a ‘magic trait’ model

of evolution [1,11] reflecting a combination of colour-based mate choice and natural selection on colour pattern through aggressive mimicry and crypsis [24,25]. It has been suggested that eight hamlet colour morphs are each aggressive mimics of different non-predatory reef fish models [23–25], plus two hamlets posited to cryptically match their environment [18,25]. In addition, the occurrence of ‘odd’ colour morphs such as the golden or masked hamlet (figure 1) that are neither cryptic nor obvious mimics suggests that hamlet colour morphs may also evolve and persist in the absence of natural selection. Here, we evaluate the potential for sexual selection alone to drive the evolution of assortative mating in the presence of gene flow using a combination of behavioural observations, individual-based simulations and population genetic analyses.

2. PAIRING OBSERVATIONS

The relative abundance of hamlet colour morphs is highly variable across reefs and regions, and can be strongly skewed, with some morphs very abundant and others very rare on the same reef [23,26,27]. In Bocas del Toro, Panama, we intensively surveyed a 25 500 m² reef and recorded nine colour morphs (figure 1), of which two were abundant ($n > 300$ individuals), one moderately abundant ($n = 21$) and six rare ($n \leq 3$). We exploited the natural differences in the relative abundance of colour morphs to investigate the dynamics of hamlet pair formation and mating success.

The first question we addressed directly using the Bocas del Toro system was the cost of assortative mating paid by rare colour morphs. Over the course of two periods of observation, we tagged the moderately abundant and rare hamlets and followed individuals in order to observe the process of pairing and mating. For 12 dusk dives on successive days in September 2009, two divers followed individuals representing the rare tan (*Hypoplectrus* sp.), golden (*Hypoplectrus gummigutta*), yellowbelly (*Hypoplectrus aberrans*) and shy (*Hypoplectrus guttavarius*) hamlets and recorded their pairing interactions. These included pairings,

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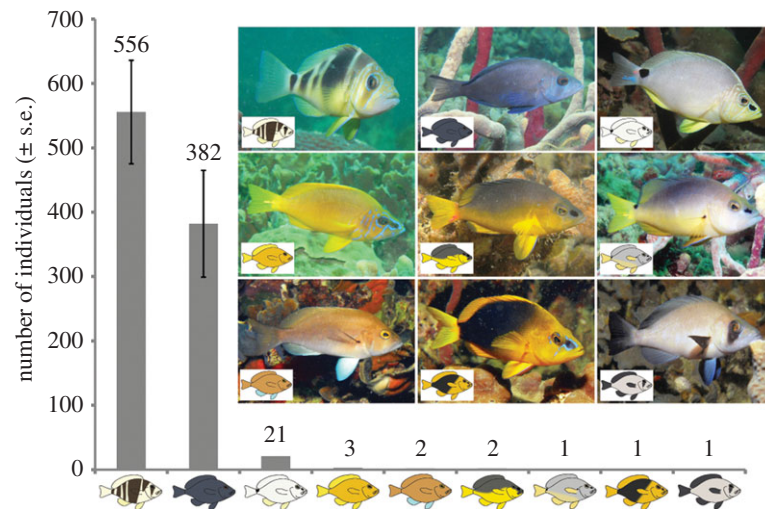


Figure 1. Abundances of nine *Hypoplectrus* colour morphs on a single 85×300 m reef in Bocas del Toro (Panama). Photographs (from upper left to lower right): barred hamlet (*Hypoplectrus puella*), black hamlet (*Hypoplectrus nigricans*), butter hamlet (*Hypoplectrus unicolor*), golden hamlet (*Hypoplectrus gummigutta*), yellowbelly hamlet (*Hypoplectrus aberrans*), tan hamlet (*Hypoplectrus* sp.), tan hamlet type 2, shy hamlet (*Hypoplectrus guttavarius*) and masked hamlet (*Hypoplectrus providencianus*). The relative abundances of the different colour morphs remained stable over the course of the study. Note the visible implant elastomer tags on the caudal fins of some of the fish. Photographs by O. Puebla.

where two individuals remain paired for at least 20 min, displaying to each other and spawning [21], as well as courtship interactions (displaying or chasing) that did not result into actual pairings. In April 2010, we repeated our observations including a subset of 10 moderately abundant butter hamlets (*Hypoplectrus unicolor*) spawning over the same area of reef. Given the very low frequencies of rare phenotypes ($\leq 0.2\%$), the expectation was that these morphs would pay a search and encounter cost relative to more abundant hamlet morphs, resulting in a reduced number of mating opportunities. Contrary to expectations, our first set of observations in September 2009 indicated that rare golden and yellowbelly hamlets did not lose time looking for a mate. Individuals would swim hundreds of metres to well-established rendezvous points on the reef where they would meet their partner every evening and spawn (electronic supplementary material, movie S1).

For example, the two adult golden hamlets on the reef paired on 12/12 evenings (individuals B and C, figure 2a); measured by mating success, the rare golden hamlets mated significantly more than the butter hamlets which—in spite of their intermediate abundance on the reef—paired on 4/12 evenings on average (figure 2c; Fisher's exact test p -value < 0.0001). Thus contrary to expectations, our observations indicate that rarity can confer a mating advantage within specific social contexts. (Clearly, rare morphs have to find each other in the first place, which may constitute a cost. But since the hamlets interact socially before becoming sexually mature, they may meet future mates before they start spawning, as observed with the juvenile golden hamlet (D) in figure 2a.) Nonetheless, but in line with expectations, we did document a significant cost to rarity in the case of the lone shy hamlet (A) on the reef. This individual mated on 0/12 evenings, significantly less than the moderately abundant butter hamlets (Fisher's exact test p -value = 0.010). The golden and shy hamlets contrast in terms of costs of assortative mating despite both colour morphs being

extremely rare, with less than 1 adult per 10 000 m^2 of reef. These observations suggest that rarity alone is not a strong predictor of pairing success, and open up new lines of inquiry regarding pairing dynamics. In particular, they challenged us to consider the formation of mating pairs within its social context, i.e. with respect to surrounding pairs and individuals.

Between observation periods, the juvenile golden hamlet (D) in September 2009 became reproductively active and the original pair (B,C) became less stable. The destabilization was manifest in two ways. First, the number of assortative mating events was reduced among golden hamlets from 12/12 evenings in September 2009 (B and C, figure 2a) to an average of 5/12 evenings in April 2010 (B–D, figure 2b). Second, two of the golden hamlets paired and mated disassortatively in April 2010, compared with zero instances of non-assortative mating in September 2009 (figure 2b). Similarly, one yellowbelly hamlet (G) disappeared from the reef between September 2009 and April 2010, and its mate (F) developed a relatively stable relationship with the lone tan hamlet (E) that had not mated during the 12 evenings of observation in September 2009. These observations suggest that both pairing success and hybridization in hamlets are dependant on the social and community matrix in which the individual is embedded. More precisely, the formation of mating pairs and their stability over time appear to depend on the composition of surrounding pairs and unmated individuals, and this context-dependent aspect of the pairing process introduces variability in pairing success.

High variability in pairing success and pair stability is also observed among the hamlets of intermediate density. One set of observations focused on butter hamlets mentioned earlier and pictured in figure 2c. A single stable pair formed between the largest two individuals (figure 2c,d); the remaining individuals spent a significant amount of time interacting (chasing and displaying),

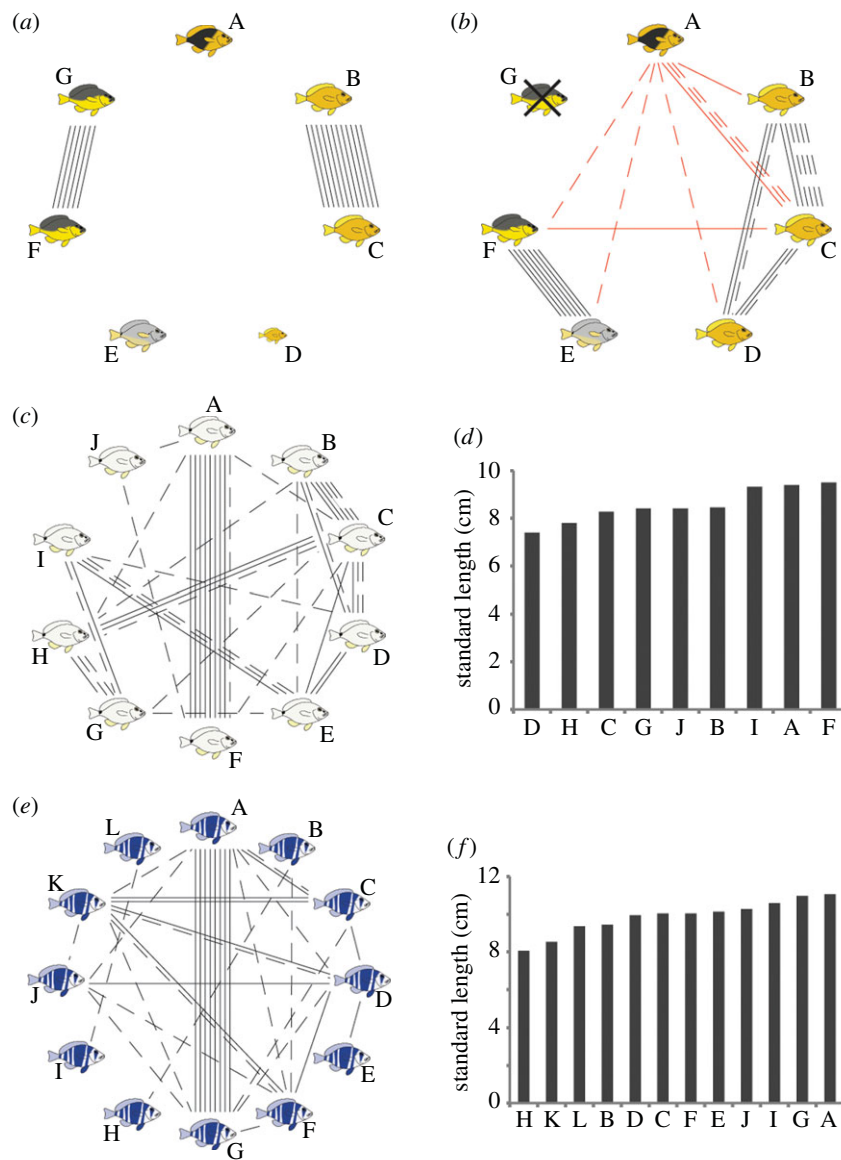


Figure 2. Pairing dynamics among *Hypoplectrus* colour morphs. Solid lines represent pairings and dashed lines courtship interactions (display or chasing). Interspecific interactions highlighted in red. (a,b) Rare morphs from Bocas del Toro (Panama) in September 2009 and April 2010, respectively (12 days observation each). One golden hamlet was a juvenile in September 2009 and one yellowbelly hamlet disappeared from the reef in April 2010. (c) Butter hamlets from Bocas del Toro, 12 days observation (April 2010). (d) Standard lengths of the butter hamlets. (e) Indigo hamlets from Carrie Bow Cay (Belize), 8 days observation (December 2009). (f) Standard lengths of the indigo hamlets.

and spawned fewer times. Extreme variation in the pairing dynamics and mating success of hamlets at intermediate densities was confirmed by a set of independent observations of the indigo hamlet (*Hypoplectrus indigo*) in Carrie Bow Cay (Belize) in December 2009 (figure 2e,f).

All-in-all, individuals involved in stable pairs mate more than individuals who are not. This is clearly illustrated in figure 2, and confirmed by the highly significant correlation between the total number of pairings per individual and the maximum number of pairings with the same partner (Spearman's $r = 0.918$ over all spawning observations, p -value < 0.0001). While long-term pairing and mating observations will be required to detail the behavioural drivers of spawning, the patterns of context-dependent pairing and pair stability reported earlier suggest that they may play a role for the evolution of assortative mating. However, the classic sexual selection theory

does not explicitly take context-dependent pairing stability into account, suggesting that a new theoretical framework may be required to better understand the evolution and maintenance of assortative mating.

3. MATCHING MODEL

The matching theory [28] in economics explicitly considers the stability of matchings between members of two groups (two-sided matching, e.g. men and women, the 'marriage problem') or members of a single group (one-sided matching, e.g. roommates, the 'roommate problem'). Hamlets fit into the one-sided category because they are simultaneous hermaphrodites. Matching theory is based on preference lists, whereby each participant ranks all potential partners according to his preferences and attempts to pair with the highest-ranking partner. A new pair will form only when both partners

benefit in terms of pairing with a preferred partner. Thus, pairing depends not only on the traits and preferences of the two individuals, but also on the traits, preferences and pairing configuration of the rest of the population.

Matching theory seems to have been largely overlooked in the speciation literature [29], and we are aware of a single theoretical study [30] that has used this approach to investigate the implications of mate choice for the evolution of assortative mating. Here, we take a matching approach to develop a model of mutual mate choice tailored to the specific case of the hamlets, and evaluate the implications of pairing dynamics for the evolution of assortative mating in the presence of gene flow.

A flowchart summarizing the model is provided in the electronic supplementary material (figure S2). Individuals are diploid simultaneous hermaphrodites and generations are discrete. Each individual is characterized by a phenotypic trait x (colour pattern) and a mating trait m that controls mating preference with respect to x (disassortative, random, assortative). x and m are controlled by N_x and N_m unlinked additive loci, respectively. All loci have two alleles (0, 1) and mutation rate μ . The phenotype x or m consists of the sum of alleles at the N_x or N_m loci rescaled to vary between 0 and 1. Each individual has also 10 unlinked neutral 'microsatellite' loci with 20 alleles and mutation rate $\mu_k = 5 \times 10^{-4}$ per generation typical of microsatellite markers [31]. Mutation follows a k -allele model.

The pairing dynamics are modelled explicitly as specified by the following algorithm: for all individuals, if $m > 0.5$, all potential mates are sorted by decreasing similarity with respect to x (similar individuals preferred, assortative mating), and if $m \leq 0.5$ all potential mates are sorted by increasing similarity with respect to x (dissimilar individuals preferred, disassortative mating). Individuals with the same similarity are listed randomly with respect to each other. Every position on the preference list is exchanged with another randomly chosen position with probability $f(m)$, where $f(m) = m$ if $m \leq 0.5$ and $1 - m$ otherwise. Thus, $f(0.5) = 0.5$ corresponds to random preferences with respect to x , and $f(0) = f(1) = 0$ corresponds to strictly disassortative and assortative preferences, respectively. Each individual (picked following a random sequence) proposes to any potential mate ranking higher than his current mate on his preference list. If the proposing individual also ranks higher in the preference list of the potential mate than his current mate, then a new pair is formed and the former mates of the two individuals become single. This procedure of proposing and accepting/rejecting is repeated for n_i iterations for the whole population.

The number of offspring produced per pair is equal to the stability of the pair, i.e. the number of iterations over which no switching of mate occurred, with all pairs producing at least t_{\min} and at most t_{\max} offspring. t_{\max} is set equal to t_{\min} for the case without stability advantage (all pairs produce the same number of offspring), and to $5 \times t_{\min}$ for the case with stability advantage. This confers at most a fivefold advantage to more stable pairs, which corresponds to the maximum difference in number of pairings between stable and unstable pairs observed in the field (figure 2).

The probability of survival to the adult stage is given by the density-dependent function $D = 1/(1 + e^{(n_o - K)/b})$, with n_o corresponding to the number of offspring, K to

the carrying capacity (2250) and b set to $K/2$. These parameter values were selected empirically to generate a population of about 1000 individuals (corresponding to the hamlet population on the reef surveyed in Bocas del Toro) with relatively small fluctuations.

A facultative source of disruptive natural selection on x is also included, with specific values of x (i.e. specific colour patterns) providing a fitness advantage (see details in electronic supplementary material). A minimum of three replicate simulations were run for each set of parameter values explored.

Three categories of simulations are illustrated in figure 3. In the absence of both stability advantage and natural selection, assortative mating does not evolve (figure 3a) and the population becomes monomorphic with respect to x after about 500 generations (figure 3b). With natural selection alone (no stability advantage), assortative mating evolves to fixation in about 300 generations (figure 3c), and the population splits into two phenotypic clusters corresponding to the two optimal phenotypes favoured by natural selection (figure 3d). This result is in line with previous 'magic trait' models, where a phenotypic trait is both under disruptive selection and used as a cue for mating [1,4–8,11,32]. Nonetheless, figure 3e establishes that pair stability alone is sufficient to drive the evolution of assortative mating, and is a new result indicating that natural selection is not required for the evolution of assortative mating in the presence of gene flow. Unlike the case with natural selection, all phenotypes (issued from mutation or standing genetic variation) are maintained in the population in this case, even when rare (figure 3f). The tendency for assortative mating to evolve in the presence of gene flow by sexual selection alone is a robust outcome in our model. It occurred for all replicate simulations of all parameter combinations tested, as long as more stable pairs have a higher fitness (see electronic supplementary material).

4. POPULATION GENETICS

The evolution of assortative mating in our model implies that all individuals strictly prefer mates similar to themselves in terms of colour pattern. Nevertheless, when no like mate is available, model conditions are such that they go down their preference list until they find a partner. This results in hybridization between colour morphs and low levels of genetic differentiation at neutral genetic markers (figure 4). Model results are consistent with the occurrence of hybridization in the field (figure 2 and electronic supplementary material, table S1), and the low genetic differences between sympatric hamlet colour morphs in nature revealed by microsatellite markers (figure 5). In addition, the apparent absence of intrinsic post-zygotic barriers to fertilization between colour morphs [26,33] and the rare occurrence of individuals with intermediate colour patterns in the field suggest that gene flow between colour morphs occurs naturally in the hamlets.

In order to evaluate the hypothesis of ongoing gene flow between colour morphs, we analysed genetic data with a three-population 'isolation with migration' model using the coalescent approach [34–37]. The analyses were performed on barred, black and butter hamlets from Bocas del Toro (Panama), and repeated on barred, black and indigo hamlets from Carrie Bow Cay (Belize), with each colour morph from each site represented by a

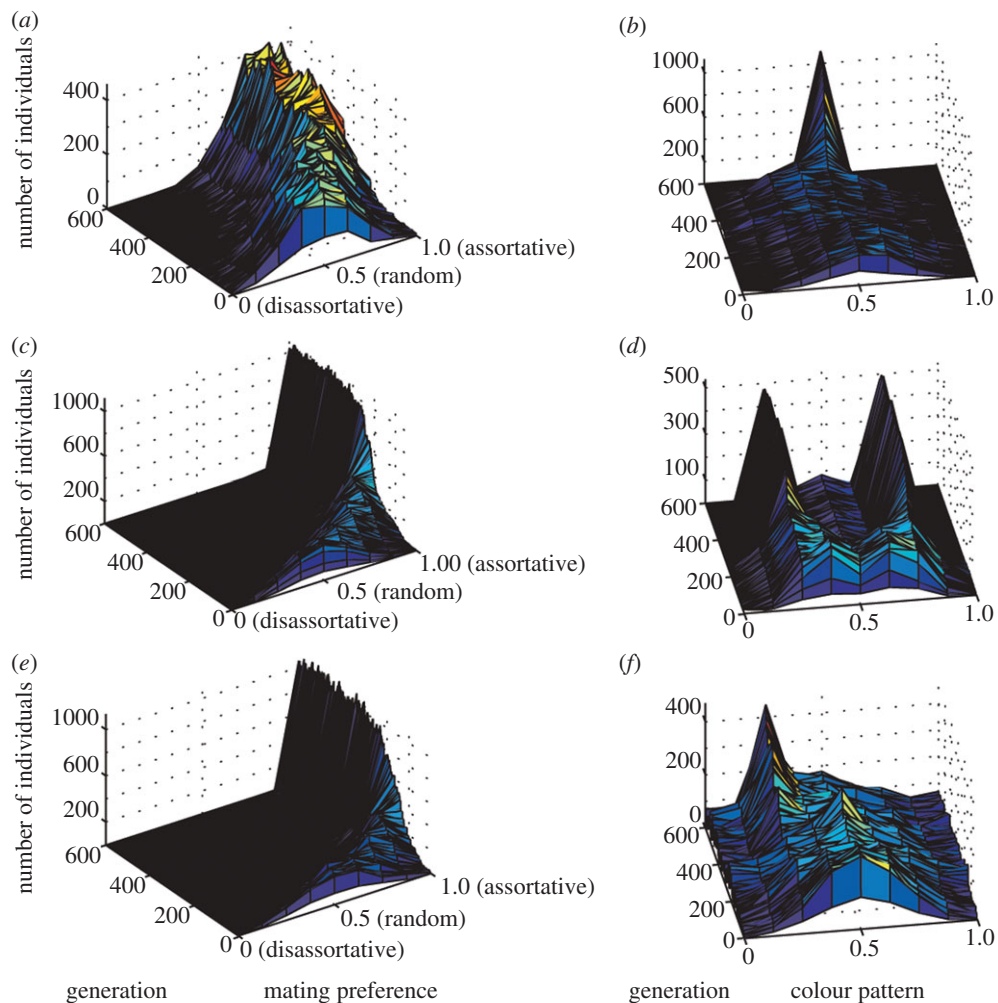


Figure 3. Illustration of three categories of simulations. (a,b) No natural selection and no stability advantage. Assortative does not evolve in this case. (c,d) Natural selection alone. Assortative mating evolves in about 300 generations, with the population splitting into two clusters corresponding to the two phenotypes favoured by natural selection. (e,f) Stability advantage alone, whereby individuals involved in more stable pairs have a higher fitness. The stability advantage alone is sufficient to drive the evolution of assortative mating. All phenotypes are maintained in this case, even when rare. $\mu = 1 \times 10^{-5}$ per generation, $N_x = N_m = 4$, and $n_i = 100$ for all simulations.

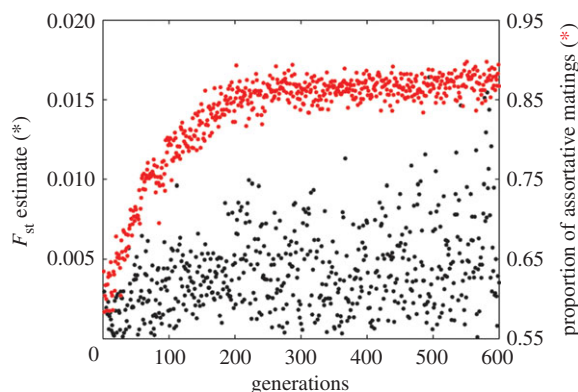


Figure 4. Assortative mating (red asterisks) and genetic differentiation (black asterisks) in the simulations without natural selection (stability advantage alone, figure 2e,f). Even when assortative mating is fixed after about 300 generations, i.e. when all individuals prefer mates with the same trait (colour pattern) as themselves, a substantial amount of hybridization (approx. 13%) is maintained, resulting in low genetic differences between morphs at neutral 'microsatellite' markers.

sample of 50 individuals genotyped at 11 microsatellite loci and sequenced for a 643 bp region of the mitochondrial cytochrome oxidase I gene (see electronic supplementary material). Likelihood-ratio tests [34,37] pointed to gene flow between sympatric morphs in both Bocas del Toro and Belize (electronic supplementary material, tables S5 and S6). In addition, a nested model with all migration parameters set to 0 was rejected with both datasets (ESS = 1.0, see IMA2 documentation for interpretation).

5. DISCUSSION

We have presented field observations, individual-based simulations and genetic analyses showing how pairing dynamics in a context of mutual mate choice can drive the evolution of assortative mating in the presence of gene flow, without invoking natural disruptive selection.

The evolution of assortative mating in our model is based on complex non-equilibrium pairing dynamics, which excludes an exhaustive analytical exploration of the parameter space. Gale & Shapley [28] showed that

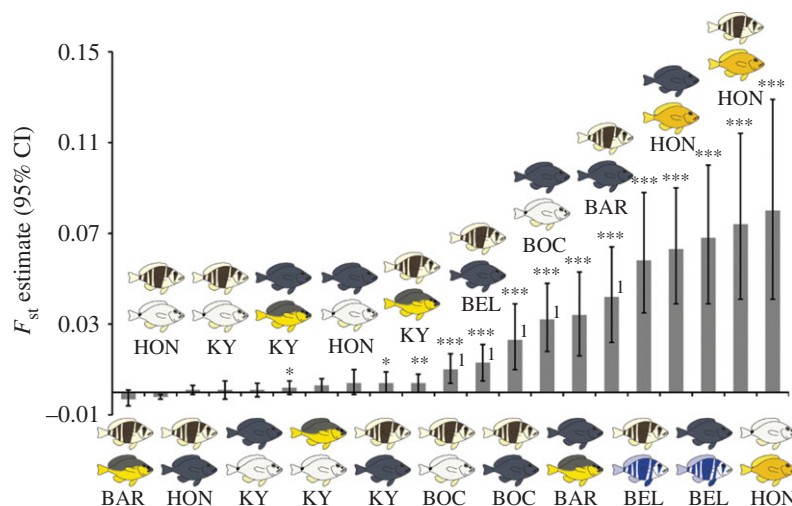


Figure 5. Genetic differentiation between pairs of sympatric *Hypoplectrus* colour morphs from Belize, Honduras, Panama and Barbados genotyped at 10 microsatellite loci (50 individuals per sample), in order of increasing genetic differentiation. F_{st} estimates less than 0 are interpreted as 0. *, **, *** F_{st} estimate significantly greater than 0 at the 0.05, 0.01 and 0.001 level, respectively, after sequential Bonferroni correction for multiple tests (G -tests). BEL, Belize; HON, Honduras; BOC, Bocas del Toro (Panama); KY, Kuna Yala (Panama); BAR, Barbados. ¹Data from previous analyses [23]. See also electronic supplementary material, tables S3 and S4.

a stable set of matchings, i.e. a situation where all pairs are stable, may not necessarily exist for the ‘roommate problem’, which is equivalent to the problem of pairing in the hamlets. Even if a stable set of matching does exist, it may be practically impossible to reach in the community context that characterizes the hamlets, given the large number of pairing possibilities. There are $(2N)!/(2^N(N!))$ ways to match $2N$ hamlets in pairs—that is, 945 and 10 395 possibilities in the situations illustrated in figure 2c,e, respectively. Given such a context, it is not surprising that many hamlets do not form stable pairs (figure 2).

An intuitive way to understand the evolution of assortative mating is to consider that stable pairs are those with matching traits and preferences, creating a correlation (linkage disequilibrium) between traits and preferences if the pair realizes a reproductive advantage. In addition, only genotypes of stable pairs for which the two members have the same trait and preference will not be disrupted by Mendelian segregation in subsequent generations, which selects for clusters of individuals with the same trait and a preference for this trait, i.e. incipient species. Almeida & de Abreu [30] proposed this mechanism in 2003 and suggested that it could apply to a wide variety of taxa, including yeasts, east-African cichlids, mammals and plants. Although our model differs from theirs in several respects (e.g. simultaneous hermaphroditism of hamlets versus separate sexes; see electronic supplementary material, table S7), we reach the same conclusion that consideration of pairing dynamics can lead to the evolution of assortative mating in the presence of gene flow, and that this is a robust outcome requiring a limited number of assumptions and no fine-tuning of parameter values.

Two fundamental assumptions of the evolutionary mechanism described here are mutual mate choice and reproductive advantage accruing to stable pairs. A stability advantage applies to all situations where remaining paired for a given amount of time is critical for reproductive success. For the hamlets, the observation that individuals involved in stable pairs minimize the

reproductive time lost to chasing and displaying and mate more than individuals who switch partners suggests that they have a reproductive advantage. The importance of spending time together to increase reproductive success applies to a variety of situations such as, e.g. long courtships, building nests or raising offspring. The stability of mating pairs is critical in these situations because reproductive success will be affected if one of the two mates decides to switch partners during this time. As for mutual mate choice, it is known to occur in a variety of taxa, including fish, birds, amphibians, insects and rotifers [38]. In addition, while mate choice is often thought of as the familiar situation where females choose males, the significance of male mate choice for the maintenance of female ornamentation is receiving increasing attention and support, even in polygynous species [38–41].

In simulations based on the matching theory model built for hamlets, exploration of the parameter space indicates that assortative mating evolves under a wide range of values (see electronic supplementary material). The number of loci controlling traits and preferences as well as initial conditions have particularly strong effects on the rate at which assortative mating evolves (electronic supplementary material, figures S4 and S5). The simulations presented in figure 3 are initiated with randomly generated genotypes. Starting with an initially monomorphic population (and a mutation rate of 1×10^{-4} per generation), assortative mating evolves to fixation in about 2000 generations (electronic supplementary material, figure S5). The evolution of assortative mating can only be as fast as the generation of variability in traits and preferences, and the simulations presented here consider a population of about a thousand individuals corresponding to the hamlet population on the reef surveyed in Bocas del Toro. The hamlet population in the wider Caribbean is spread across tens of thousands of such reefs interconnected by larval dispersal [42], and thus the large population size of hamlets across their distribution provides ample opportunity for the generation of variability.

Even when assortative mating evolves to fixation in the model, gene flow between morphs still occurs when individuals go down their preference list and do not encounter an available mating partner of their colour pattern. Hybridization between colour morphs results, maintaining gene flow among colour morphs and sustaining low genetic differentiation at neutral genetic markers (figure 4). Accordingly, the mechanism described here is best regarded as a framework for the evolution of assortative mating rather than a speciation mechanism *sensu stricto*. Other processes not included in the model may contribute to further increase the strength of assortative mating. This seems to be the case in the hamlets, where both our field observations and previous aquaria experiments [26] indicate that individuals are reluctant to mate with unlike mates, even when they have no other alternative, and will often choose not to mate at all in such situations. This is consistent with the lower hybridization rates (0–5%) and higher F_{st} estimates (0–0.08) observed in nature for hamlets versus those in the model (5–15 and 0–0.05%, respectively, over all parameter values tested for a population size of 1000 individuals or more).

Natural or sexual selection alone can drive the evolution of assortative mating in our model. A fundamental difference between the two is that only optimal phenotypes (and a few hybrids) are favoured under natural selection (figure 3d), whereas any phenotype can be maintained by sexual selection (figure 3f). Figure 3f also indicates that the relative abundances of the different morphs can change through time, with some morphs becoming abundant and others rare, and that rare morphs do not go extinct. Thus, the pairing dynamics alone can provide a relatively simple explanation not only for (i) the evolution of assortative mating in the presence of gene flow, (ii) the potentially rapid diversification into several morphs, (iii) ongoing hybridization and low genetic differences between morphs, (iv) but also for the differences in relative abundances between morphs, and (v) the persistence of rare morphs.

In conclusion, we suggest that the matching theory in the context of mutual mate choice is an overlooked framework for understanding the evolution of assortative mating. Considering pairing dynamics explicitly in theoretical and empirical studies of mate choice has the potential to increase our understanding of the evolution of assortative mating and its implications for speciation in the presence of gene flow.

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