

Imprinting sets the stage for speciation

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Sexual imprinting—a phenomenon in which offspring learn parental traits and later use them as a model for their own mate preferences—can generate reproductive barriers between species¹. When the target of imprinting is a mating trait that differs among young lineages, imprinted preferences may contribute to behavioural isolation and facilitate speciation^{1,2}. However, in most models of speciation by sexual selection, divergent natural selection is also required; the latter acts to generate and maintain variation in the sexually selected trait or traits, and in the mating preferences that act upon them³. Here we demonstrate that imprinting, in addition to mediating female mate preferences, can shape biases in male–male aggression. These biases can act similarly to natural selection to maintain variation in traits and mate preferences, which facilitates reproductive isolation driven entirely by sexual selection. Using a cross-fostering study, we show that both male and female strawberry poison frogs (*Oophaga pumilio*) imprint on coloration, which is a mating trait that has diverged recently and rapidly in this species⁴. Cross-fostered females prefer to court mates of the same colour as their foster mother, and cross-fostered males are more aggressive towards rivals that share the colour of their foster mother. We also use a simple population-genetics model to demonstrate that when both male aggression biases and female mate preferences are formed through parental imprinting, sexual selection alone can (1) stabilize a sympatric polymorphism and (2) strengthen the trait–preference association that leads to behavioural reproductive isolation. Our study provides evidence of imprinting in an amphibian and suggests that this rarely considered combination of rival and sexual imprinting can reduce gene flow between individuals that bear divergent mating traits, which sets the stage for speciation by sexual selection.

Sexual selection can drive rapid divergence in mating signals and preferences, which may then lead to behavioural isolation among phenotypic variants and thereby facilitate speciation². Although the potential for speciation by sexual selection has long been acknowledged, theoretical work has identified two major challenges for this mechanism to occur when there is gene flow between incipient species: (i) the association between a genetic mating trait and a genetic preference can easily be broken down by recombination⁵ and (ii) assortative mating often degrades genetic variation in mating traits and preferences, which eliminates the polymorphisms that provide the basis for future divergence^{6,7}. Sexual imprinting—a phenomenon in which offspring learn parental phenotypes as the basis of their own mate preferences—presents a solution to the problem of recombination^{1,8}. Because offspring inherit their mating trait from the parent or parents that they imprint on, the association between trait and preference reforms anew in each generation. The second challenge, of achieving stable polymorphisms, can be resolved by incorporating divergent ecological selection that acts directly on mating traits³ (known as ‘magic traits’⁹) or mating preferences (such as sensory drive¹⁰). However, in these scenarios natural selection is arguably a more important driver of speciation than is sexual selection, because of the contribution of natural selection to the origin and maintenance of trait and preference variation.

Assortative male–male competition that is mediated by the same mating signal that female preferences act on can also generate divergent

selection through negative-frequency-dependent selection (known as ‘rare male advantage’¹¹), and may provide an alternative mechanism by which sexual selection, on its own, can stabilize a polymorphism^{6,12}. In this scenario, the mating trait inherently becomes a magic trait, which affects both divergence and reproductive isolation solely via sexual selection and without the need for a pleiotropic ecological effect. This mechanism may be widespread because sexually selected traits are often used for both mate choice and intrasexual aggression¹³. Furthermore, ‘species recognition’ (stronger behavioural responses towards conspecifics) often involves behavioural biases in both sexes¹⁴. Mathematical models^{6,15} that have incorporated male–male competition as the source of balancing selection have assumed that individuals are more competitive or aggressive towards individuals that share their own phenotype. However, as has been demonstrated for mating biases⁸, the mechanisms that shape aggressive behavioural biases are diverse (for example, genetic versus plastic)^{12,16,17}, and the evolutionary trajectories that result from biases generated through these various mechanisms remain largely unexplored.

Here we tested for imprinted behaviours in a species that shows evidence of recent, rapid divergence in a sexually selected trait on which both female mate preferences and male aggressive biases act. In and around the Bocas del Toro archipelago of Panama, *O. pumilio* exhibits extreme, heritable polymorphisms in coloration^{18,19}. Although most of the colour variation occurs among isolated island populations, there are a few areas of sympatric polymorphism^{18,20,21} (Fig. 1). The populations of *O. pumilio* in this region have probably experienced periods of vicariance and reconnection due to the rise and fall of sea levels; however, comparisons of neutral genotypic variation with phenotypic variation suggest a major role of selection in the rapid divergence of colours⁴. Despite evidence that colour can be aposematic in this species²², colour variants appear to incur similar risks of predation^{23,24}. This suggests that differential predation—the most obvious candidate for natural selection—may have had a relatively minor role in shaping coloration in *O. pumilio*, compared to other poison frog species²⁵. By contrast, sexual selection seems to be a strong driver of the evolution of colour differences in *O. pumilio*. In general, females prefer to court males that share their own colour morph over males with novel colour morphs^{21,26}. Although males have not been studied as extensively, they are more aggressive towards their own colour morph²⁷. Because no post-zygotic incompatibilities appear to exist¹⁹, these divergent, colour-biased sexual behaviours probably represent the most salient reproductive barrier among the colour morphs.

We tested the hypothesis that imprinting shapes both female mate preferences (referred to as ‘sexual imprinting’^{1,8}) and male aggression biases (referred to as ‘rival imprinting’¹⁶) among three colour morphs of *O. pumilio* using a rearing experiment (Fig. 2). The biparental care exhibited by this species provides ample opportunity for tadpoles to observe adult colours. Adult males tend their eggs, and females transport tadpoles to waterholes on their backs and later feed their begging tadpoles with their own unfertilized eggs throughout larval development²⁸. We tested for colour biases in female mate preference and male–male aggression of laboratory-reared, socially naive frogs that were either purebred (with both parents being of the same colour), crossbred (with each parent of a different colour) or cross-fostered (raised by

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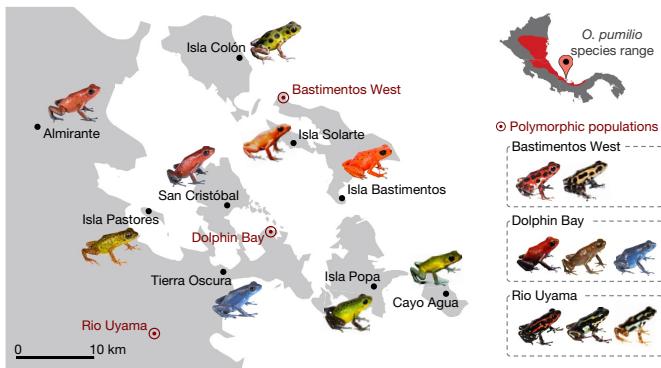


Fig. 1 | Map of the Bocas del Toro archipelago, showing the diversity of colour and pattern among populations of *O. pumilio*. Top right, species range of *O. pumilio* across Nicaragua, Costa Rica and Panama. The location pin indicates the Bocas del Toro archipelago. Left, colour-monomorphic populations are labelled with a solid dot and an exemplar of the colour morph is shown next to the respective dot. Sympatric colour-polymorphic populations are labelled with a circled dot, and exemplars of the colour variants are shown on the right. Colour variation at Bastimentos West²⁰ is discrete, whereas colour variation at Dolphin Bay^{21,27} and Rio Uyama¹⁸ is continuous. We used three allopatric colour morphs in the rearing experiment: those from Isla Popa, Isla Bastimentos and Tierra Oscura. Photographs by V. Prémel, J. P. Lawrence, S. A. Echeverri, I. J. Wang and Y.Y. Map data 2018, Google.

foster parents of a different colour than the biological parents) (Fig. 2a, b). With one exception, males and females from the three rearing treatments spent similar amounts of time with the stimulus individuals (that is, were similarly responsive) (Supplementary Information). Overall, the behavioural patterns of courtship (in females) and rival aggression (in males) of the frogs were similar in all three treatments (Fig. 2c, d, Extended Data Table 1). Purebred offspring of either sex biased their interactions towards males of their own colour over those of another colour, which established the fact that laboratory-reared frogs exhibit similar colour-biased behaviours to those of wild *O. pumilio*. Cross-fostered offspring showed a bias towards the colour of their foster parents over that of their biological parents, which suggests that imprinting is more influential than genetics in shaping these colour-mediated behaviours. Additionally, crossbred offspring of either sex biased their interactions towards males that shared the colour of the offspring's mother over males that shared the colour of the father, which suggests that interaction with the mother is more influential than interaction with the father in determining colour biases. We hypothesize that *O. pumilio* learn the coloration of their mother during the tadpole stage, and use it as a template for mate preference and rival aggression biases in adulthood. Our results suggest that maternal imprinting may be the key mechanism that mediates the colour-assortative behaviours that are seen among recently diverged colour morphs of *O. pumilio*.

We further explored the evolutionary implications of these imprinted behaviours using a simple population-genetics model, in which imprinting shapes both female mate preferences and male aggression biases. In nature, the genetic architecture of polymorphic mating traits appears highly variable (ranging from simple Mendelian to highly polygenic inheritance); a commonly observed pattern is one in which a single mutated allele leads to a marked phenotypic change (such as in the influence of MC1R on animal melanin coloration^{29,30}). Although studies of the genetic architecture of coloration in *O. pumilio* are in their infancy, there are examples that suggest both polygenic^{19,27} and simple Mendelian inheritance of colour²⁰ (Fig. 1). As an initial step, we used a diploid model that incorporates a mating trait governed by a diallelic Mendelian locus (with dominant and recessive alleles), on which both female mate preferences and male aggression biases act. In our model, both female preferences and male aggression biases are learned by imprinting on the mating trait of a parent (as we found evidence for in *O. pumilio*). We defined

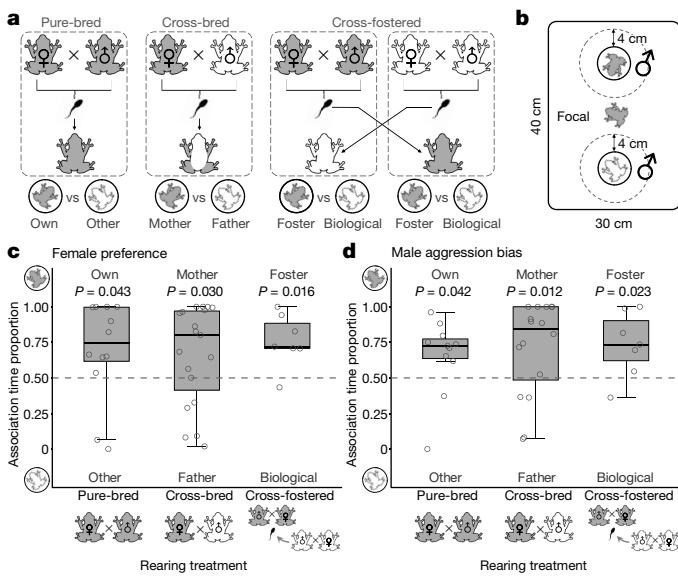


Fig. 2 | *O. pumilio* rearing experiment. **a**, Experimental design. Purebred denotes offspring raised by their biological parents, both of which were of the same colour morph. Crossbred denotes offspring raised by their biological parents, each of which was a different colour morph. Cross-fostered denotes offspring raised by foster parents (which had a colour morph different to that of the biological parents). The coloration of offspring from crossbred pairs has previously been described¹⁹. **b**, Experimental apparatus used in the behavioural assays. During behavioural observations, the two stimulus males were confined under clear plastic domes, and the focal individual could move freely in the arena. **c, d**, The proportions of the total time that female (**c**) and male (**d**) offspring spent with one of the two stimulus-male phenotypes during the behavioural assay. Purebred, own colour/(own colour + other colour); crossbred, colour of the mother/(colour of the mother + colour of the father); cross-fostered, colour of the foster parent/(colour of the foster parent + colour of the biological parent). Values above the dashed line ($y = 0.5$) indicate a preference or aggression bias that is consistent with maternal imprinting. P values were based on one-sided, one-sample permutational *t*-tests (Extended Data Table 1, Supplementary Information). Bold lines indicate medians. Boxes enclose 25th to 75th percentiles. Error bars enclose data range, excluding outliers. Dots are data points; dots that are vertically outside the error bars are outliers (below quartile 1, minus $1.5 \times$ the interquartile range or above quartile 3, plus $1.5 \times$ the interquartile range). The sample sizes (number of focal frogs) were purebred male, 11; purebred female, 12; crossbred male, 16; crossbred female, 19; cross-fostered male, 7; and cross-fostered female, 7.

preference strength (α) and aggression-bias strength (β) such that, upon encountering a conspecific, females are $1 + \alpha$ times as likely to mate with the imprinted phenotype than with the alternative phenotype and males are $1 + \beta$ times as aggressive towards the imprinted phenotype in male–male competition. We assumed that males that receive more aggression from other males are less likely to establish a territory (and thereby have reduced reproductive success¹⁴), and calculated the effective frequencies of male genotypes that enter the mating pool (from which the females choose their mates).

We compared results from two versions of the model that differ only in whether the imprinting was on the mating trait of the mother or of father. The two models yield qualitatively similar conclusions. We were interested in identifying the conditions under which both mating-trait phenotypes coexist stably in our model. We found that the stability of such a polymorphism is dependent on the relative strengths of the female mate preference (α) and male aggression bias (β) (Fig. 3a). Although initial allele frequencies affect the maintenance of trait variation, an initially rare trait allele (frequency = 0.0001) can increase in frequency to reach stable polymorphism across a wide range of preference and aggression strengths (Extended Data Fig. 1). In addition, incomplete imprinting (when not every individual imprints

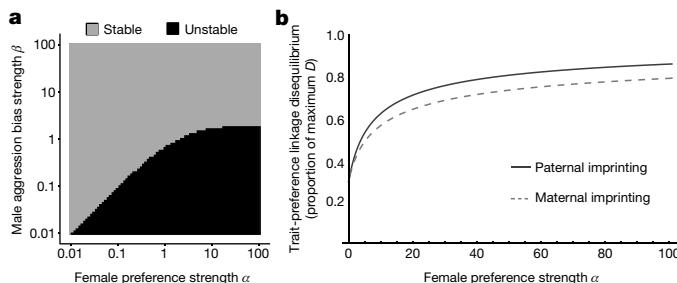


Fig. 3 | Polymorphism stability and associations between trait and preference. **a**, Stability of polymorphic equilibria for combinations of α and β from 0.01 to 100, with a step size of $10^{0.05}$. Axes are on logarithmic scales. Maternal and paternal imprinting models yield visually identical results. Across the range of parameters that we tested, the strength of β required to maintain a stable polymorphism increases non-linearly with α . **b**, Association between mating trait locus T and female mate preference ‘locus’ P (measured as the phenogenotypic linkage disequilibrium between the trait genotype and the preference phenotype, presented as a proportion of the maximum coefficient of the linkage disequilibrium (D); Supplementary Information) at the polymorphic equilibrium (trait 1 phenotype frequency = 0.5) increases with α . Note that the stability of such a polymorphic equilibrium depends on the relative strength of α and β (**a**). The trait–preference linkage disequilibrium, expressed in terms of α (it is independent of β), is described in the Supplementary Information.

successfully on the phenotype of the parent) will linearly decrease the realized strength of both α and β , which results in a simulation outcome similar to that seen in Fig. 3a with smaller values on the x and y axes (Supplementary Information). Mechanistically, through sexual imprinting, mating trait frequencies determine the behaviour frequencies of offspring (both female mate preferences and male aggression biases); these behaviours then serve as the source of sexual selection that acts on the mating trait in the next generation. Therefore, selection generated by both female preference and male aggression is frequency-dependent, and delayed by one generation. Consistent with previous studies^{3,31}, imprinted female mate preference exerts positive-frequency-dependent selection that favours the more-common mating trait allele and would on its own drive that allele to fixation. By contrast, male aggression bias generates negative-frequency-dependent selection that counters the pull towards fixation⁶, which generates a stable polymorphism in the mating trait when the aggression bias is sufficiently strong (the grey area of Fig. 3a). In this basic model, the frequency of the mating trait phenotype always stabilizes at 0.5. However, when we extend the model to allow asymmetry in the strengths of selection (that is, when the values of α and β vary with the phenotype on which the individual imprinted), the trait polymorphism can stabilize at broad range of frequencies (Extended Data Figs. 2, 3, Supplementary Information).

We also evaluated the association between the mating trait and mate preference at the polymorphic equilibrium. Divergent mating and aggressive behaviours will not generate two completely isolated mating groups unless female mate preference is absolute (females never mate with the non-preferred phenotype ($\alpha = \infty$)). Instead, we looked for parameter space in which a positive association between trait and behaviour was formed, as this indicates reduced gene flow between the two trait variants—which could set the stage for speciation⁵. We found that the formation of this positive association between trait and behaviour (calculated as the phenogenotypic linkage disequilibrium between the behavioural phenotype and the trait genotype) (Methods, Supplementary Information) is independent of β and increases with α (Fig. 3b). However, as α increases the minimum β required for a stable polymorphism also increases (Fig. 3a). Consequently, pre-mating behavioural isolation is most likely to evolve via this mechanism when female preference is strong—but it cannot do so without being accompanied by a male aggression bias that is strong enough to maintain a stable polymorphism during the process. Along with simulations that

explore the robustness of these findings to other initial frequencies of the mating trait, our model suggests that maternally imprinted sexual behaviours may begin to generate reproductive isolation when allopatric colour morphs come into secondary contact (as has probably occurred many times, owing to changes in sea level), when parapatric populations contribute new trait variants by infrequent migrations—or even among colour morphs that arise in sympatry, as long as sexual selection is strong.

Sexual selection is likely to facilitate speciation when divergent traits are accompanied by divergent sexual behaviours. We provide empirical evidence that suggests that maternal sexual imprinting on a heritable, polymorphic mating trait mediates both female mate choice and male–male competition in a poison frog. We also demonstrate using a mathematical model that imprinted aggression biases towards rival males can act in concert with sexually imprinted female mate preferences to maintain a stable polymorphism and reduce gene flow between divergent mating phenotypes in sympatry. Thus, parental imprinting provides a plausible and effective mechanism through which a sexually selected trait and the behaviours that act on it may co-diverge as a result of sexual selection alone, leading to reduced gene flow between sympatric lineages and setting the stage for speciation.

Online content

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- Irwin, D. E. & Price, T. Sexual imprinting, learning and speciation. *Heredity* **82**, 347–354 (1999).
- Ritchie, M. G. Sexual selection and speciation. *Annu. Rev. Ecol. Evol. Syst.* **38**, 79–102 (2007).
- Servedio, M. R. & Boughman, J. W. The role of sexual selection in local adaptation and speciation. *Annu. Rev. Ecol. Evol. Syst.* **48**, 85–109 (2017).
- Brown, J. L., Maan, M. E., Cummings, M. E. & Summers, K. Evidence for selection on coloration in a Panamanian poison frog: a coalescent-based approach. *J. Biogeogr.* **37**, 891–901 (2010).
- Felsenstein, J. Skepticism towards Santa Rosalia, or why are there so few kinds of animals? *Evolution* **35**, 124–138 (1981).
- van Doorn, G. S., Dieckmann, U. & Weissing, F. J. Sympatric speciation by sexual selection: a critical reevaluation. *Am. Nat.* **163**, 709–725 (2004).
- Arnegard, M. E. & Kondrashov, A. S. Sympatric speciation by sexual selection alone is unlikely. *Evolution* **58**, 222–237 (2004).
- Verzijden, M. N. et al. The impact of learning on sexual selection and speciation. *Trends Ecol. Evol.* **27**, 511–519 (2012).
- Servedio, M. R., Van Doorn, G. S., Kopp, M., Frame, A. M. & Nosil, P. Magic traits in speciation: ‘magic’ but not rare? *Trends Ecol. Evol.* **26**, 389–397 (2011).
- Boughman, J. W. How sensory drive can promote speciation. *Trends Ecol. Evol.* **17**, 571–577 (2002).
- Seehausen, O. & Schlüter, D. Male–male competition and nuptial-colour displacement as a diversifying force in Lake Victoria cichlid fishes. *Proc. R. Soc. Lond. B* **271**, 1345–1353 (2004).
- Dijkstra, P. D. & Border, S. E. How does male–male competition generate negative frequency-dependent selection and disruptive selection during speciation? *Curr. Zool.* **64**, 89–99 (2018).
- Berglund, A., Bisazza, A. & Pilastro, A. Armaments and ornaments: an evolutionary explanation of traits of dual utility. *Biol. J. Linn. Soc.* **58**, 385–399 (1996).
- Grether, G. F., Peiman, K. S., Tobias, J. A. & Robinson, B. W. Causes and consequences of behavioral interference between species. *Trends Ecol. Evol.* **32**, 760–772 (2017).
- Mikami, O. K., Kohda, M. & Kawata, M. A new hypothesis for species coexistence: male–male repulsion promotes coexistence of competing species. *Popul. Ecol.* **46**, 213–217 (2004).
- Hansen, B. T. & Stagsvold, T. Rival imprinting: interspecifically cross-fostered tits defend their territories against heterospecific intruders. *Anim. Behav.* **65**, 1117–1123 (2003).
- Verzijden, M. N., Korthof, R. E. M. & Ten Cate, C. Females learn from mothers and males learn from others. The effect of mother and siblings on the development of female mate preferences and male aggression biases in Lake Victoria cichlids, genus *Mbipia*. *Behav. Ecol. Sociobiol.* **62**, 1359–1368 (2008).
- Summers, K., Cronin, T. W. & Kennedy, T. Variation in spectral reflectance among populations of *Dendrobates pumilio*, the strawberry poison frog, in the Bocas del Toro Archipelago, Panama. *J. Biogeogr.* **30**, 35–53 (2003).

19. Dugas, M. B. & Richards-Zawacki, C. L. A captive breeding experiment reveals no evidence of reproductive isolation among lineages of a polytypic poison frog. *Biol. J. Linn. Soc.* **116**, 52–62 (2015).
20. Richards-Zawacki, C. L., Wang, I. J. & Summers, K. Mate choice and the genetic basis for colour variation in polymorphic dart frog: inferences from a wild pedigree. *Mol. Ecol.* **21**, 3879–3892 (2012).
21. Yang, Y., Richards-Zawacki, C. L., Devar, A. & Dugas, M. B. Poison frog color morphs express assortative mate preferences in allopatry but not sympatry. *Evolution* **70**, 2778–2788 (2016).
22. Saporito, R. A., Zuercher, R., Roberts, M., Kenneth, G. & Donnelly, M. A. Experimental evidence for aposematism in the dendrobatid poison frog *Oophaga pumilio*. *Copeia* **2007**, 1006–1011 (2007).
23. Hegna, R. H., Saporito, R. A. & Donnelly, M. A. Not all colors are equal: predation and color polyporphism in the aposematic poison frog *Oophaga pumilio*. *Evol. Ecol.* **27**, 831–845 (2013).
24. Richards-Zawacki, C. L., Yeager, J. & Bart, H. P. S. No evidence for differential survival or predation between sympatric color morphs of an aposematic poison frog. *Evol. Ecol.* **27**, 783–795 (2013).
25. Rojas, B. Behavioural, ecological, and evolutionary aspects of diversity in frog colour patterns. *Biol. Rev. Camb. Philos. Soc.* **92**, 1059–1080 (2017).
26. Maan, M. E. & Cummings, M. E. Female preferences for aposematic signal components in a polymorphic poison frog. *Evolution* **62**, 2334–2345 (2008).
27. Yang, Y., Dugas, M. B., Sudekum, H. J., Murphy, S. & Richards-Zawacki, C. L. Male–male aggression is unlikely to stabilize a poison frog polymorphism. *J. Evol. Biol.* **31**, 457–468 (2018).
28. Dugas, M. B. Simple observations with complex implications: what we have learned and can learn about parental care from a frog that feeds its young. *Zool. Anz.* **273**, 192–202 (2018).
29. Uy, J. A. C., Moyle, R. G., Filardi, C. E. & Cheviron, Z. A. Difference in plumage color used in species recognition between incipient species is linked to a single amino acid substitution in the melanocortin-1 receptor. *Am. Nat.* **174**, 244–254 (2009).
30. Hoekstra, H. E., Hirschmann, R. J., Bundey, R. A., Insel, P. A. & Crossland, J. P. A single amino acid mutation contributes to adaptive beach mouse color pattern. *Science* **313**, 101–104 (2006).
31. Verzijden, M. N., Lachlan, R. F. & Servedio, M. R. Female mate-choice behavior and sympatric speciation. *Evolution* **59**, 2097–2108 (2005).

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METHODS

No statistical methods were used to predetermine sample size. The experiments were not randomized and investigators were not blinded to allocation during experiments and outcome assessment.

Rearing experiment. We reared socially naive *O. pumilio* individuals from frogs in a breeding colony, haphazardly selecting from three contrasting colour morphs (red, blue and green) (Fig. 1, Supplementary Information). The rearing experiment included three treatments: (i) purebred (offspring raised by their biological parents, both of which were of the same colour morph); (ii) crossbred (offspring raised by their biological parents, each of which was a different colour morph); and (iii) cross-fostered (offspring raised by foster parents that were of a colour different from that of the biological parents) (Fig. 2a). Offspring were removed from rearing enclosures within 24 h of reaching Gosner stage 46 (complete metamorphosis³²) and transferred to a separate enclosure, and were kept physically and visually isolated from other frogs in the colony until the behavioural assay, which was conducted after the individual had reached sexual maturity (10–12 months after metamorphosis). We tested for colour-based behavioural biases in the male and female offspring using a two-way choice test (Fig. 2b) among males of contrasting colours (purebred, own colour versus another colour; crossbred, colour of the mother versus colour of the father; cross-fostering, colour of biological parents versus colour of foster parents). Sample sizes were 11 (purebred male), 12 (purebred female), 16 (crossbred male), 19 (crossbred female), 7 (cross-fostered male) and 7 (cross-fostered female).

Behavioural assay. Two-way choice experiments were carried out to assess the behaviour of offspring towards males of various phenotypes: the focal individual could move freely in an arena, and the two stimulus males were confined under transparent plastic domes (Fig. 2b). Unrelated adult males were used as stimulus males, and stimulus-male pairs were matched for size and mass but differed in colour. Details of the experimental setup and protocol are described in the Supplementary Information. We quantified (i) association time (defined as the cumulative time that the focal frog spent in each of the 4-cm (about 2 body lengths) interaction zones that surrounded the dome of each male) and (ii) approaches (defined as the number of times that the focal frog oriented towards and entered each interaction zone).

Statistical analyses. To test for female mate preference and male aggression biases, we calculated the proportions of the total association time that male and female offspring spent with one of the two male phenotypes presented to them (purebred, own colour / (own colour + other colour); crossbred, colour of the mother/(colour of the mother + colour of the father); and cross-fostered, colour of the foster parent/(colour of the foster parent + colour of the biological parent)). We tested the hypothesis that these proportions of association time were >0.5 for the three rearing treatments and two sexes separately, using one-tailed one-sample permutation *t*-tests (Extended Data Table 1). We also ran the same analyses with ratios of the number of approaches to each stimulus phenotype as a second, supplementary confirmation of the pattern (Extended Data Table 2). Additional details in given in the Supplementary Information.

Population-genetics model. We developed a diploid model with discrete, non-overlapping generations. The mating trait is governed by a single diallelic locus T with a dominant allele (T_1) and a recessive allele (T_2) (T_{11} denotes two copies of the dominant allele, T_{22} denotes two copies of the recessive allele and T_{12} denotes heterozygosity). We also used a behaviour ‘locus’ P (actually a phenotype) to denote the trait on which the individuals have imprinted. P is inherited via maternal or paternal imprinting, and governs both female mate preference and male aggression biases, being either P_1 (biased towards trait 1) or P_2 (biased towards trait 2). Each individual can therefore be described by combination of a phenotype and a genotype (a ‘phenogenotype’) that contains a diploid mating trait locus T (the genotype) and a haploid behaviour ‘locus’ P (the phenotype). The frequencies of the six phenogenotypes $T_{11}P_1$, $T_{12}P_1$, $T_{22}P_1$, $T_{11}P_2$, $T_{12}P_2$ and $T_{22}P_2$ are designated x_1, x_2, x_3, x_4, x_5 and x_6 , respectively.

In our model, the lifecycle consists of male–male competition, female mate choice, reproduction and imprinting. During male–male competition, males are $1 + \beta_k$ times as aggressive towards the imprinted mating-trait phenotype (k). For example, the total aggression received by mating phenotype 1 is $A_1 = (1 + \beta_1)p_1 + p_2$ where p_1 and p_2 represent the frequencies of trait-1-biased and trait-2-biased males, respectively ($p_1 = x_1 + x_2 + x_3$, $p_2 = x_4 + x_5 + x_6$). The fitness (ω) of males with mating phenotype k decreases as the total aggression received increases, such that $\omega_k = 1 - s_k$ where $s_k = A_k / \sum_k A_k$. Because the behavioural bias of males is not relevant in the portion of the lifecycle that comes after male–male competition, we pool males with the P_1 and P_2 phenotypes and calculate only the frequencies of the three male genotypes. T_{11} , T_{12} and T_{22} males are designated $x_{1,m}$, $x_{2,m}$ and $x_{3,m}$, respectively (subscript m denotes male). The effective frequency of males with mating genotype i that enter the mating pool after male competition is therefore:

$$x_{i,m}^* = \frac{\omega_k x_{i,m}}{\sum_i \omega_k x_{i,m}}$$

in which $k = 1$ when $i = 1$ or 2, and $k = 2$ when $i = 3$. There is no competition between females (denoted by subscript f) in this model, so the frequency of female phenogenotypes that enter the mating pool is $x_{j,f}^* = x_{j,f}$ where the six female phenogenotypes $T_{11}P_1$, $T_{12}P_1$, $T_{22}P_1$, $T_{11}P_2$, $T_{12}P_2$ and $T_{22}P_2$ are designated $x_{1,f}$, $x_{2,f}$, $x_{3,f}$, $x_{4,f}$, $x_{5,f}$ and $x_{6,f}$ respectively.

After male competition, the females choose their mates according to the behaviour ‘locus’ P, such that upon encountering a male, females are $1 + \alpha_k$ times as likely to mate with males that possess the imprinted mating phenotype k (following a previous publication³³). Thus, the frequency of mating between each combination of male genotype i and female phenogenotype j is:

$$F_{i,j} = \frac{x_{i,m}^* x_{j,f}^* (1 + d_{i,j} \alpha_k)}{\sum_i x_{i,m}^* (1 + d_{i,j} \alpha_k)}$$

where $d_{i,j} = 1$ if the female behaviour ‘locus’ P matches the male trait phenotype T ($i = 1$ or 2 with $j = 1, 2$ or 3, and $i = 3$ with $j = 4, 5$ or 6) and $d_{i,j} = 0$ otherwise, and where $k = 1$ when $j = 1, 2$ or 3, and $k = 2$ when $j = 4, 5$, or 6. The denominator normalizes the frequencies to ensure that females have equal mating success (strict polygyny).

Reproduction and imprinting happen after mating, at which point the phenotype frequencies of the resulting zygotes are calculated. The mating trait locus T is genetically inherited with Mendelian segregation. The phenotypic ‘locus’ P is obtained either by maternal or by paternal imprinting. All offspring with a trait-1 (T_{11} or T_{12}) parent (mother for maternal printing or father for paternal printing) are P_1 individuals, and all offspring with a trait 2 (T_{22}) parent are P_2 individuals.

Details of the recursion equations and numerical analyses are described in the Supplementary Information. The recursion equations were not solvable analytically, and were analysed by estimating numerical solutions using Mathematica³⁴ and using deterministic simulations. We considered two conditions to be important for assessing progress towards the evolution of reproductive isolation³¹: (i) whether the polymorphic equilibrium of mating trait T is stable (Fig. 3a) and (ii) whether the mating traits (T) and the behaviours (P) were associated (that is, in phenogenotypic linkage disequilibrium between the mating trait genotype and the behavioural phenotype) at the polymorphic equilibrium (Fig. 3b). We also assessed the extent to which the potential for achieving a stable polymorphic state depended upon the starting frequencies of the alleles (Extended Data Fig. 1). Our basic model (discussed in the main text) assumed symmetrical selective strengths on both mating phenotypes ($\alpha_1 = \alpha_2$ and $\beta_1 = \beta_2$). We also analysed scenarios in which the strength of the behavioural biases differs between the P_1 and P_2 individuals ($\alpha_1 \neq \alpha_2$ and $\beta_1 \neq \beta_2$; Supplementary Information). The asymmetries allow the polymorphic equilibrium to stabilize at a wide range of phenotype frequencies (from 0 to 1), but the qualitative conclusions reported above regarding polymorphism stability and the evolution of linkage disequilibrium between the mating trait and the behaviours were robust (Extended Data Figs. 2, 3, Supplementary Information). All analyses were performed using Mathematica.

Reporting Summary. Further information on research design is available in the Nature Research Reporting Summary linked to this paper.

Data availability

The datasets generated during and/or analysed during the current study have been deposited in Figshare (<https://doi.org/10.6084/m9.figshare.9628406>).

Code availability

Code files for statistical analysis (R) and mathematical models (Mathematica) have been deposited in Figshare (<https://doi.org/10.6084/m9.figshare.9628406>).

32. Gosner, K. L. A simplified table for staging anuran embryos larvae with notes on identification. *Herpetologica* **16**, 183–190 (1960).
33. Kirkpatrick, P. Sexual selection and the evolution of female mate choice. *Evolution* **36**, 1–12 (1982).
34. Wolfram Research. Mathematica 11.1. <https://reference.wolfram.com/legacy/language/v11.1/> (2018).

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and export permission for this study. This work complied with IACUC protocols (STRI no. 2007-17-12-15-07 and Tulane no. 0382).

Author contributions C.L.R.-Z. designed the rearing experiment and collected data, and Y.Y. carried out statistical analyses, for the breeding experiment. Y.Y. and M.R.S. performed the theoretical research. Y.Y. drafted the manuscript, and M.R.S. and C.L.R.-Z. contributed to writing and revision.

Competing interests The authors declare no competing interests.

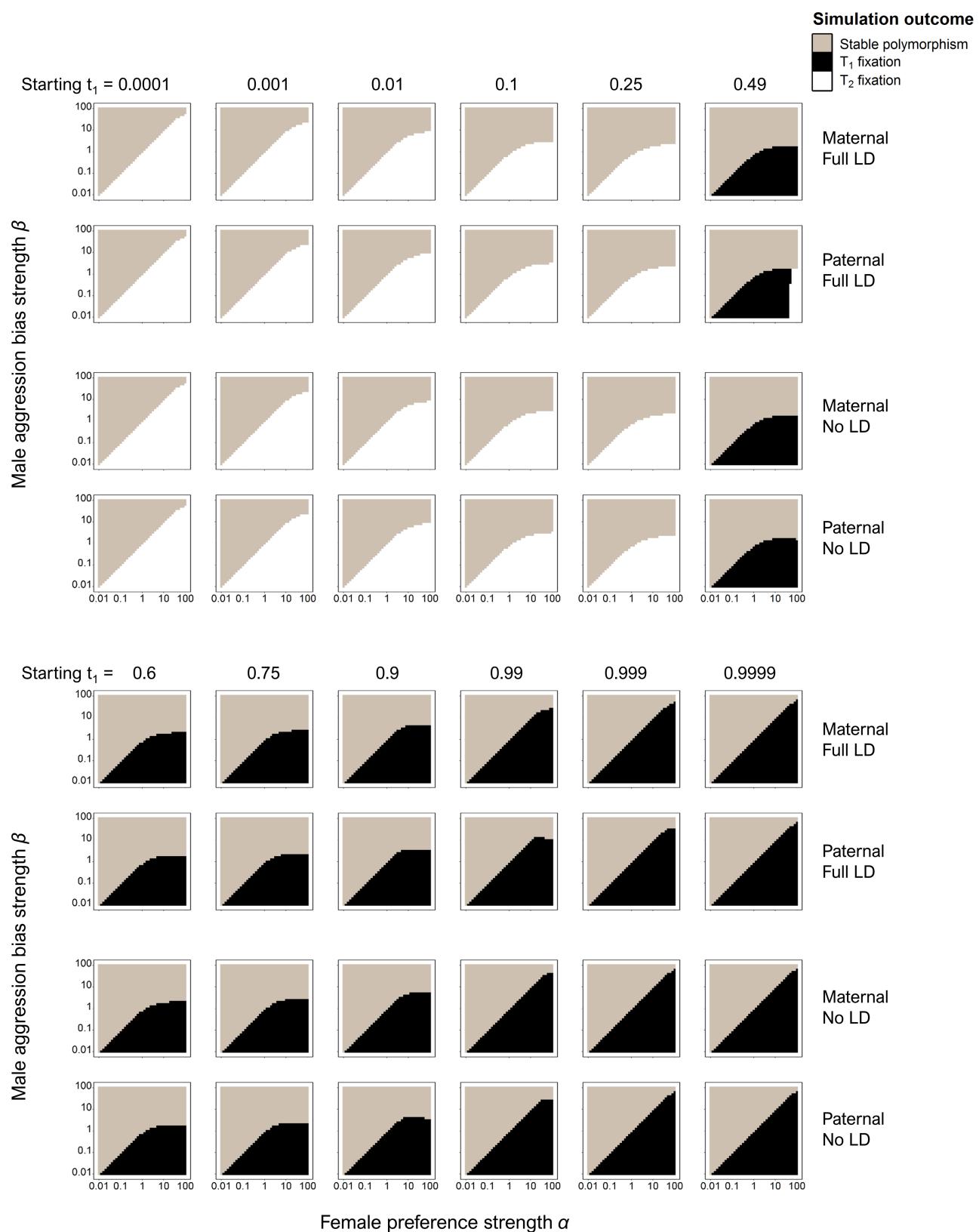
Additional information

Supplementary information is available for this paper at <https://doi.org/10.1038/s41586-019-1599-z>.

Correspondence and requests for materials should be addressed to Y.Y.

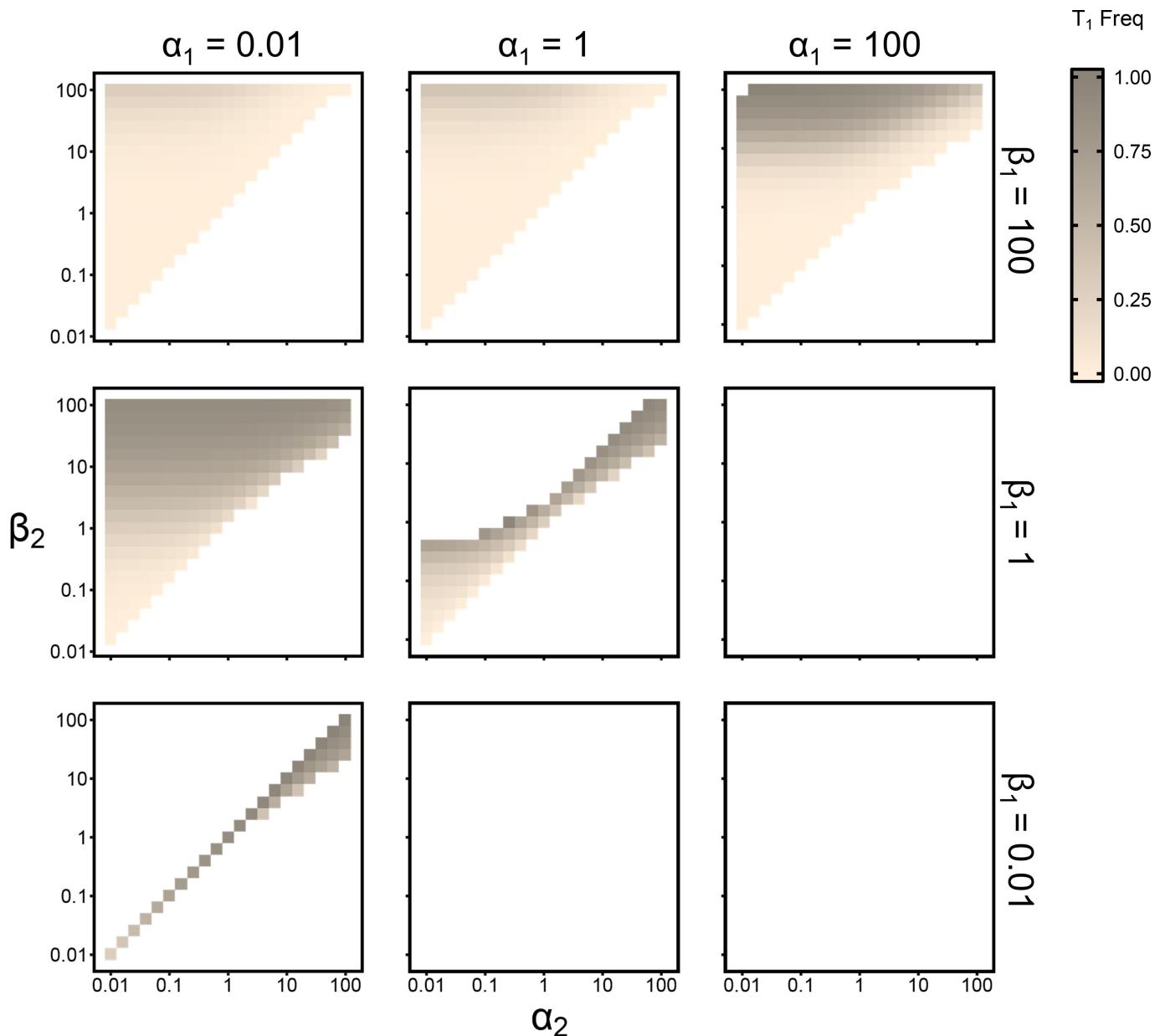
Peer review information *Nature* thanks Peter Dijkstra, Leithen M'Gonigle and Machteld Nicolette Verzijden for their contribution to the peer review of this work.

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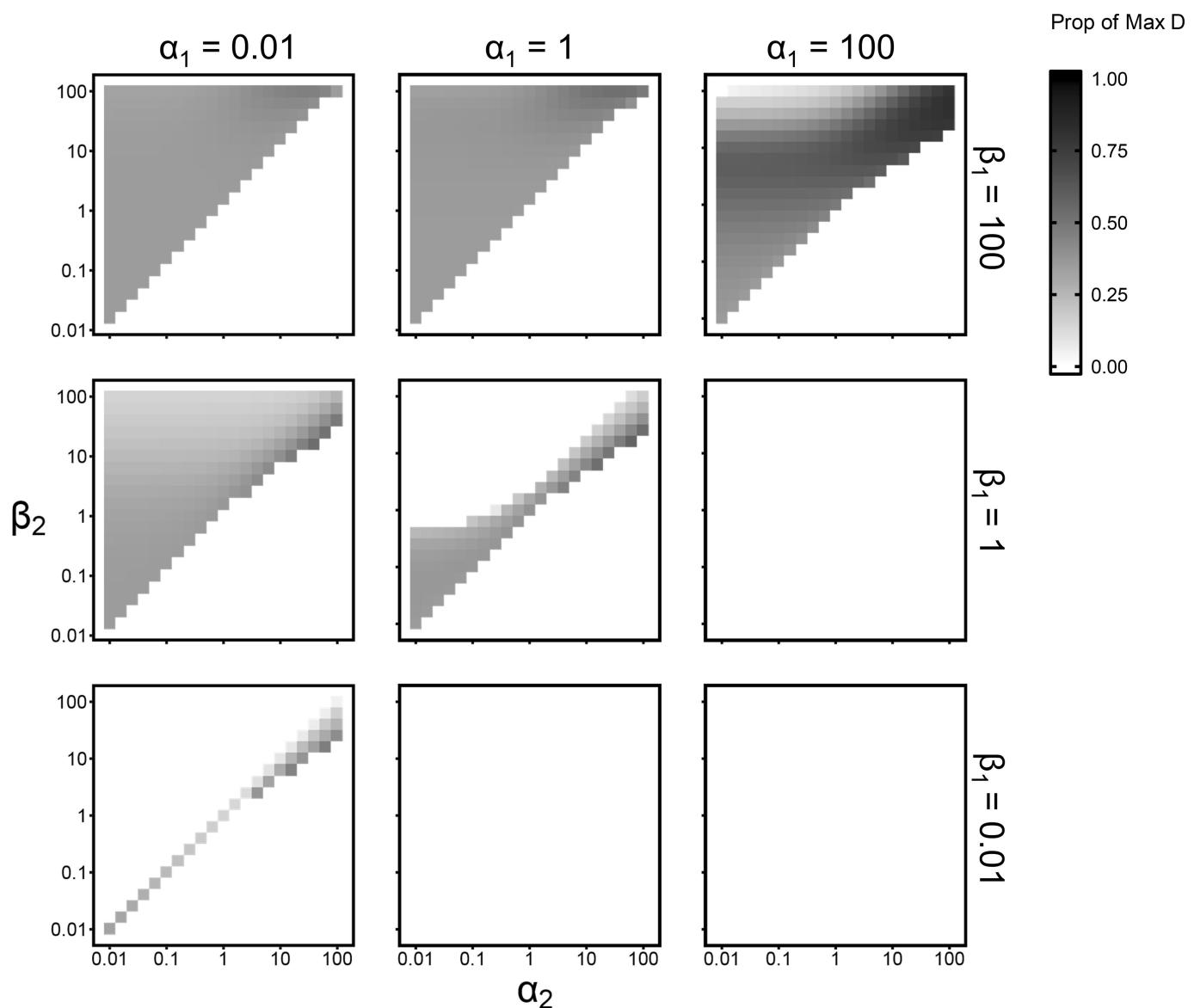
Extended Data Fig. 1 | Effects of starting frequency. Simulations of 2,000 generations demonstrate the effects of the starting frequencies of alleles on the maintenance of polymorphism. Each panel represents a combination of a particular starting frequency for T_1 (t_1 , labelled on top), association between mating trait and behaviour (phenogenotypic linkage

disequilibrium (LD) between the trait genotype and the behavioural phenotype, labelled on the right) (Supplementary Information), and types of imprinting (maternal or paternal, labelled on the right). Within each panel, we ran every combination of α and β from 0.01 to 100, with a step size of $10^{0.1}$. Axes are on logarithmic scales.



Extended Data Fig. 2 | Frequency of the trait-1 phenotype at stable polymorphic equilibrium. In this extended model, the value of α and β varies with the phenotype on which the individual imprinted (Supplementary Information). Frequency of the trait-1 phenotype includes T_{11} and T_{12} individuals ($x_1 + x_2 + x_4 + x_5$). Each panel represents a particular combination of α_1 and β_1 from the set {0.01, 1, 100}, labelled on the top and the right. Within each panel, we ran every combination of α_2

and β_2 from 0.01 to 100, with a step size of $10^{0.2}$. Axes are on logarithmic scales. The white area in each panel is the parameter space in which no stable polymorphism can be found. The frequency of the trait-1 phenotype at polymorphic equilibrium for a given combination of α_k and β_k is slightly different between the models of maternal and paternal imprinting (<0.1, not shown).



Extended Data Fig. 3 | D_{cor} at stable polymorphic equilibrium. In this extended model, the value of α and β varies with the phenotype that the individual imprinted on (Supplementary Information). Trait-behaviour linkage disequilibrium between the trait genotype and the behavioural phenotype (D_{cor} , calculated as $D/\sqrt(p_1 p_2 t_1 t_2)$) at the stable polymorphic equilibrium. Each panel represents a particular combination of α_1 and β_1

from the set {0.01, 1, 100}, labelled on the top and the right. Within each panel, we ran combinations of α_2 and β_2 from 0.01 to 100, with a step size of $10^{0.2}$. Axes are on logarithmic scales. The figure presents results from maternal imprinting models. Overall, the paternal imprinting models produced higher D_{cor} at polymorphic equilibrium than did the maternal imprinting models, but the differences were very small (<0.1, not shown).

Extended Data Table 1 | Proportions of association time for the three groups of rearing treatment

Proportion of time spent with		Female				Male			
		N	Median ± IQR	Cohens' D	p	N	Median ± IQR	Cohens' D	p
Pure-bred	Biological parents' color	12	0.74 ± 0.38	0.544	0.043	11	0.73 ± 0.14	0.587	0.042
Cross-bred	Mother's color	19	0.80 ± 0.56	0.467	0.030	16	0.85 ± 0.51	0.642	0.012
Cross-fostered	Foster parents' color	7	0.72 ± 0.18	1.409	0.016	7	0.73 ± 0.28	1.021	0.023

P values and effect sizes (Cohen's *D*) were based on one-sided one-sample permutational *t*-tests testing the proportions of association time against 0.5 separately, in the three rearing treatments and two sexes (purebred, own colour/other colour; crossbred, colour of the mother/colour of the father; cross-fostered, colour of the foster parent/colour of the biological parent) (Supplementary Information). A summarized version that shows only the *P* values is shown in Fig. 2c, d.

Extended Data Table 2 | Proportions of approaches for the three groups of rearing treatment

	Proportion of approaches toward	Female				Male			
		N	Median ± IQR	Cohens' D	p	N	Median ± IQR	Cohens' D	p
Pure-bred	Biological parents' color	12	0.71 ± 0.35	0.454	0.073	11	0.67 ± 0.08	0.418	0.113
Cross-bred	Mother's color	19	0.50 ± 0.17	0.485	0.026	16	0.71 ± 0.54	0.636	0.013
Cross-fostered	Foster parents' color	7	0.75 ± 0.25	0.941	0.047	7	0.67 ± 0.17	0.936	0.039

P values and effect sizes (Cohen's *D*) were based on one-sided one-sample permutational *t*-tests testing the proportions of approaches against 0.5 separately, in the three rearing treatments and two sexes (purebred, own colour/other colour; crossbred, colour of the mother/colour of the father; cross-fostered, colour of the foster parent/colour of the biological parent) (Supplementary Information).

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Give P values as exact values whenever suitable.
- For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
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- Estimates of effect sizes (e.g. Cohen's d , Pearson's r), indicating how they were calculated

Our web collection on [statistics for biologists](#) contains articles on many of the points above.

Software and code

Policy information about [availability of computer code](#)

Data collection

Population genetics modeling was done in Mathematica 11.1. Mathematica code and the output csv files of simulations are deposited at <https://doi.org/10.6084/m9.figshare.9628406>. Data collection for the rearing experiment does not involve programming.

Data analysis

Simulation results from Mathematica was output as csv files and graphed in R 3.5.1. Data analyses for the rearing experiment was done in R 3.5.1. Raw data and the analysis code are deposited at <https://doi.org/10.6084/m9.figshare.9628406>.

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Code and simulation results of population genetics models; raw data and statistical analyses code are deposited at <https://doi.org/10.6084/m9.figshare.9628406>

Field-specific reporting

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- Life sciences Behavioural & social sciences Ecological, evolutionary & environmental sciences

Ecological, evolutionary & environmental sciences study design

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Study description	The study consists of two parts: 1. Population genetic models: Discrete generation, rival and sexual imprinting on a diploid, Mendelian inherited sexual trait 2. Rearing experiment: factorial design. Testing color biases in the female mate preference and male-male aggression of socially naïve frogs that were either pure-bred (both parents of the same color), cross-bred (each parent a different color), or cross-fostered (raised by foster parents of a different color than the biological parents). Sample sizes were: pure-bred male = 11; pure-bred female = 12; cross-bred male = 16; cross-bred female = 19; cross-fostered male = 7; cross-fostered female = 7.
Research sample	We conducted our experiment in a laboratory colony, using three allopatric, differently colored <i>Oophaga pumilio</i> lineages from Bocas del Toro, Panama: red (Tranquilo Bay, Isla Bastimentos: 9°15.080' N, 82°80.433' W), green (Punta Laurel, Isla Popa: 9°80.260' N, 82°70.391' W) and blue (Shark Hole, Aguacate peninsula: 9°12.047' N, 82°12.049' W). The species shows evidence of recent, rapid divergence in a sexually selected trait on which both female mate preferences and male aggressive biases act. The three lineage we chose show divergence in neutral genetic markers, but no evidence of intrinsic post-zygotic isolation. Male and female frogs were captured in the field in February 2008 and housed first at the Bocas del Toro Field Station of the Smithsonian Tropical Research Institute (STRI) and later at Tulane University. We tested socially naïve frogs (see above for experimental design) produced in the colony with the age range of 221-893 days (from metamorphosis) when tested (days to sexual maturity vary considerably among individuals). The exact age of each individual is reported in the data deposited at https://doi.org/10.6084/m9.figshare.9628406 .
Sampling strategy	Rearing experiment: Our sample sizes are low because we are using a non-model organism with a low survival rate and long time to reproductive maturity. We collected as many samples as possible with the resources we had. <i>Oophaga pumilio</i> breed year round, but egg, tadpole and juvenile mortality is high (in the wild and in our colony). For those that survive, it takes ~1 year for them to reach sexual maturity. It took us many years to reach the sample size we have.
Data collection	Behavioral experiments: approaches and association time of the focal individual were timed and recorded during the assay and later entered electronically to an csv file. A. Devar and C. L. Richards-Zawacki assayed the individuals and recorded the data. The original datasheets will be kept at University of Pittsburgh.
Timing and spatial scale	Data was collected from February, 2008 to December, 2012. The behavioral assays were performed whenever the focal individual from the rearing experiment reached sexual maturity. There was no gap in sampling period.
Data exclusions	No data from the rearing experiment was excluded from the analyses. Not applicable to population genetic models.
Reproducibility	We did not attempt to redo the experiment due to constraints working with a non-model organism with a long generation time. We describe the protocol in as much detail as we can in the manuscript (in supplemental information)
Randomization	New tadpoles from same-color pairs were assigned to the pure-bred and cross-fostered treatment randomly. (Cross-bred offspring came from pairs of different color morphs)
Blinding	The experimenters were not aware of the focal individual's treatment group during the behavioral assay

Did the study involve field work? Yes No

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Methods	
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Animals and other organisms

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Laboratory animals

Three *Oophaga pumilio* lineages from Bocas del Toro, Panama: red (Tranquilo Bay, Isla Bastimentos: 9°15.080' N, 82°80.433' W),

Laboratory animals	green (Punta Laurel, Isla Popa: 9°80.260' N, 82°70.391' W) and blue (Shark Hole, Aguacate peninsula: 9°12.047' N, 82°12.049' W). All individuals assayed had reached sexual maturity. Males were assayed for aggression bias, and females for mate preference. Age range of the animals when assayed was 221-893 days (from metamorphosis). The exact age of each individual is reported in the data deposited at https://doi.org/10.6084/m9.figshare.9628406 .
Wild animals	The study did not involve wild animals.
Field-collected samples	The study did not involve samples collected from the field.
Ethics oversight	The procedures of the study followed the Animal Behavior Society Guidelines for the Use of Animals in Research, the legal requirements of the United States of America and the Republic of Panama as well as all institutional guidelines. The Panamanian National Authority for the Environment (ANAM) provided research and export permission for this study. This work complied with Institutional Animal Care and Use Committee (IACUC) protocols (Smithsonian Tropical Research Institute No. 2007-17-12-15-07 and Tulane University No. 0382).

Note that full information on the approval of the study protocol must also be provided in the manuscript.