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Materials and Methods

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References

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On the Origin of Species by Natural and Sexual Selection

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Ecological speciation is considered an adaptive response to selection for local adaptation. However, besides suitable ecological conditions, the process requires assortative mating to protect the nascent species from homogenization by gene flow. By means of a simple model, we demonstrate that disruptive ecological selection favors the evolution of sexual preferences for ornaments that signal local adaptation. Such preferences induce assortative mating with respect to ecological characters and enhance the strength of disruptive selection. Natural and sexual selection thus work in concert to achieve local adaptation and reproductive isolation, even in the presence of substantial gene flow. The resulting speciation process ensues without the divergence of mating preferences, avoiding problems that have plagued previous models of speciation by sexual selection.

Even as we commemorate the 150th anniversary of Darwin's *On the Origin of Species* (1), discussion continues on whether speciation is adaptive (that is, driven by selection) and to what extent it is inhibited by gene flow (2–7). Ecological conditions can induce natural selection for local adaptation (2, 8), but disruptive or diversifying selection is usually not sufficient for speciation if individuals can migrate between habitats. In such cases, a mating structure has to emerge that strongly reduces hybridization between ecologically specialized populations (3–5).

Sexual selection is likely to play a pivotal role in shaping such a mating structure during incipient speciation (9) and has been suggested to induce speciation by causing the divergence of mating preferences between two emerging species

(9–13). Yet speciation due to diverging mating traits is controversial, because existing theoretical models can explain the divergence of mating preferences only under conditions that are rarely met in nature (10, 13, 14). Moreover, the models tend to rely on Fisherian sexual selection to generate reproductive isolation. Fisher's runaway process of sexual selection involves preferences for arbitrary ornaments that reflect nothing but attractiveness (15). The runaway process could thus potentially evolve in different directions, allowing the divergence of preferences during speciation. However, mating preferences are generally not arbitrary but act on ornaments that indicate genetic or phenotypic quality (15–17), providing choosy individuals with either direct benefits or good genes for their offspring (15, 18–20). The adaptive directionality of mate choice based on such indicator traits makes it difficult to conceive how this process could lead to the divergence of preferences between two nascent species.

By means of the following scenario, we will demonstrate that the divergence of mating preferences is not required for sexual selection to contribute to speciation. We consider a patchy environment that imposes contrasting selection pressures on an ecological character like a food-exploitation strategy (Fig. 1). We assume that across all habitats, intermediate ecological strategies, on average, do worse than specialist strat-

egies optimizing the use of one of the habitats. Accordingly, natural selection is stabilizing within habitats but disruptive at the level of the entire population.

Individual-based computer simulations [based on Levene's "soft-selection" model (21), also see supporting online material (SOM)] that implement this ecological scenario highlight the overpowering effect of gene flow (Fig. 2A). Although disruptive selection removes individuals with intermediate phenotypes from the population, such individuals are created anew every generation as a result of migration between the habitats and recombination between different specialist genotypes. This process prevents the population from splitting into two locally adapted species, unless disruptive selection is unusually strong.

Having observed that ecological disruptive selection per se is not sufficient to result in speciation, we next consider an ornament, such as a plumage characteristic, that is expressed in a condition-dependent manner (15, 22, 23). Individuals adapted to the local environment are likely to be in a better condition, allowing them to develop brighter plumage than individuals that are less well adapted (24). Thus, by virtue of its condition-dependent expression, the ornament functions as an indicator for the degree of local adaptation (25–27).

Assuming that the ornament is expressed in males and that females are the choosy sex, one would expect females to evolve a preference for elaborate ornamentation, thereby using the information on local adaptation revealed by the male's ornament (26). Simulations that allow for the evolution of a preference and condition-dependent ornamentation [following (17); also see SOM] confirm this expectation (Fig. 2C). Starting from a randomly mating population ($p = t = 0$ at generation 0), female choosiness (p) evolved along with a concurrent increase in the resources invested by males in their ornament (t) to signal their ecological performance. The evolutionary process is driven by sexual selection and fueled by rare mutations introducing variation in female choosiness and male ornamentation. In our model, mating with a locally adapted partner is beneficial to a

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female because it increases, on average, the probability that her offspring will have an optimal phenotype in one of the habitat types and, thus, the highest fitness when selection is disruptive. Such preference for a locally adapted partner is even more advantageous when offspring are more likely to end up in the same habitat as the parents (for example, when individuals are philopatric to some degree) or exert matching habitat choice on the basis of their ecological phenotype (28).

Once mate choice has evolved, sexual selection acts alongside disruptive ecological selection to disfavor intermediate ecological phenotypes. This strengthens assortative mating with respect to the ecological strategy, reducing the rate of interbreeding between specialists for different hab-

itats. In the rare event that habitat specialists do interbreed, sexual selection effectively removes their sons from the mating pool, as hybrid males will be of poor quality in either habitat, produce less attractive ornaments, and fail to attract females. Thus, mate choice based on an indicator of local adaptation enhances reproductive isolation between habitat specialists and should therefore increase the likelihood of speciation. Indeed, the added effect of sexual selection allows the population to split into two locally adapted specialist types (Fig. 2, B and C), whereas natural selection alone merely supports the maintenance of a broad unimodal distribution of phenotypes (Fig. 2A). Replicate simulations show that the waiting time to speciation is variable, but in all cases, the pop-

ulation splits quickly and irreversibly after female choosiness has increased beyond a critical level (fig. S1). A calculation of the selection gradients on the mating characters (see SOM) reveals that these features result from a positive feedback between the effectiveness of sexual selection and ecological divergence. Selection for increased choosiness is initially weak, but as the ecological phenotype distribution changes from unimodal to bimodal, quality differences between the males become more pronounced, providing increased benefits to choosiness (fig. S2).

To further assess the contribution of sexual selection to the speciation process, we ran simulations with and without sexual selection, systematically varying the migration rate (m) and the

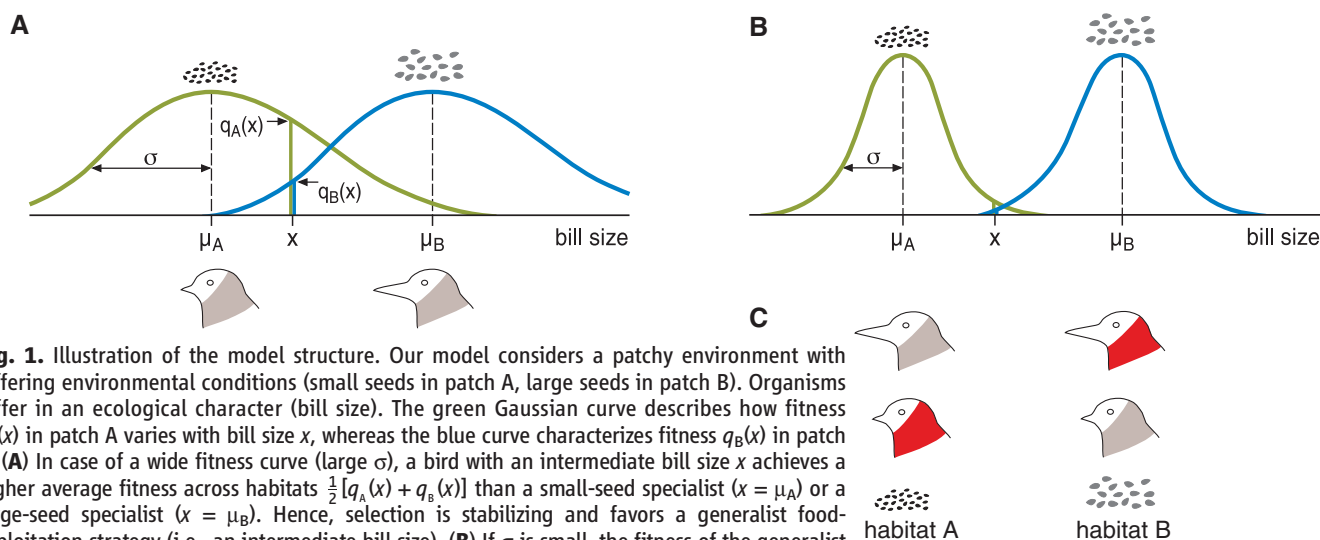


Fig. 1. Illustration of the model structure. Our model considers a patchy environment with differing environmental conditions (small seeds in patch A, large seeds in patch B). Organisms differ in an ecological character (bill size). The green Gaussian curve describes how fitness $q_A(x)$ in patch A varies with bill size x , whereas the blue curve characterizes fitness $q_B(x)$ in patch B. **(A)** In case of a wide fitness curve (large σ), a bird with an intermediate bill size x achieves a higher average fitness across habitats $\frac{1}{2}[q_A(x) + q_B(x)]$ than a small-seed specialist ($x = \mu_A$) or a large-seed specialist ($x = \mu_B$). Hence, selection is stabilizing and favors a generalist food-exploitation strategy (i.e., an intermediate bill size). **(B)** If σ is small, the fitness of the generalist strategy is very low. Selection is disruptive, favoring the two specialist food-exploitation strategies. **(C)** The colored collar represents a sexual ornament that is expressed in a condition-dependent manner. For the same allocation of resources to the ornament, small-billed birds can produce a more attractive (red) ornament in the small-seed patch A (labeled “habitat A” in the figure), whereas large-billed birds can produce a more attractive ornament in the large-seed patch B (labeled “habitat B”). Hence, the ornament functions as an indicator of local adaptation.

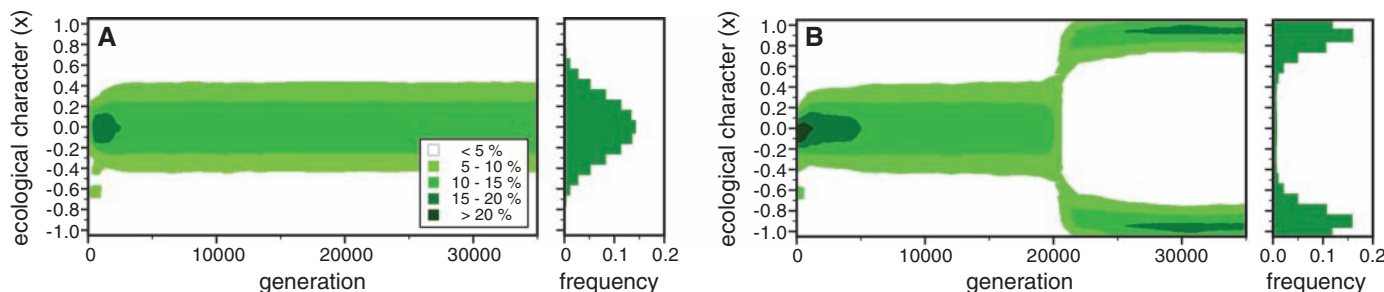


Fig. 2. Example simulation. Sexual selection on a trait signaling male quality can cause reproductive isolation between two ecologically specialized populations when natural selection alone cannot. **(A)** In the absence of sexual selection ($\alpha = 0$), a population subject to disruptive natural selection ($\mu_A = 1, \mu_B = -1, \sigma = 0.8, m = 0.75$; see SOM) evolves a broad distribution of ecological trait values around $x = 0$ (the equilibrium frequency distribution of ecological characters is shown to the right). **(B)** Under the same conditions as in (A), but with sexual selection ($\alpha = 5.0$), the population splits into two ecological specialists, as a result of the evolution of a costly female mating preference p [shown by the red line in (C)] for a male ornament. The ornament reflects a costly male investment t [blue line in (C)] and the degree of the male’s adaptation to local conditions. Error bars in (C) denote the SD of p and t to indicate the standing genetic variation in these traits.

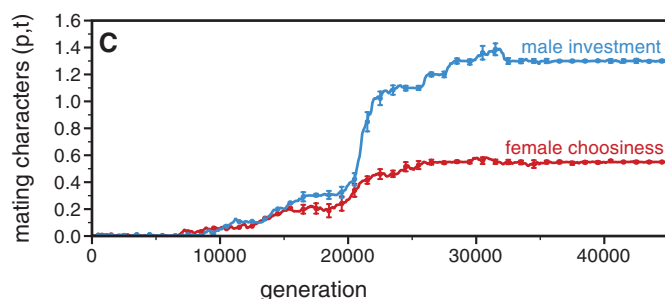
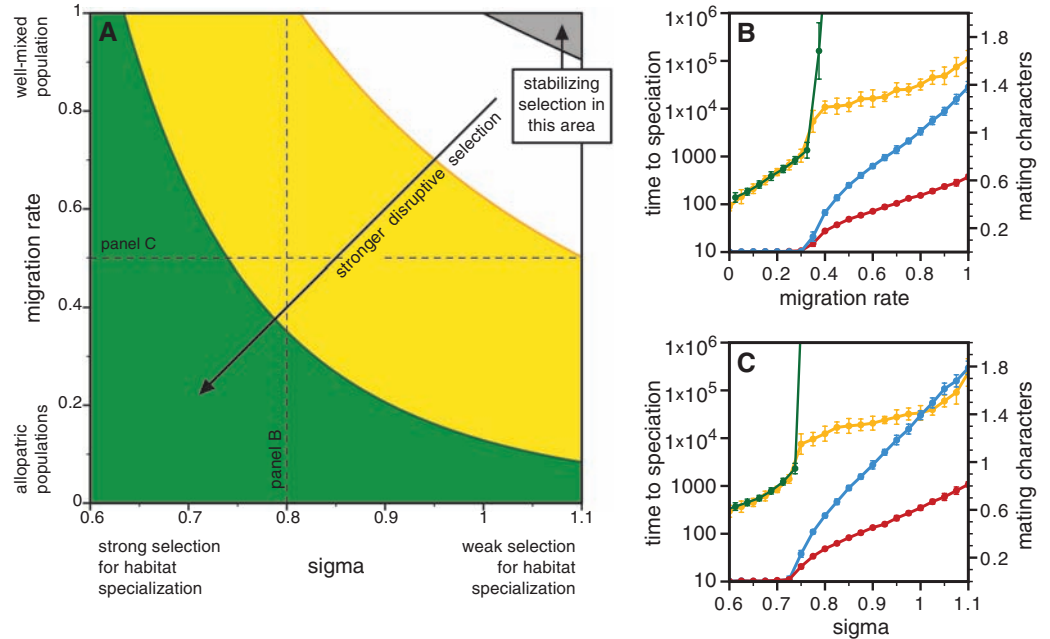


Fig. 3. Effect of ecological parameters on the rate of speciation. **(A)** Without sexual selection, ecological disruptive selection must be strong to induce speciation (green area). When mate choice reinforces ecological disruptive selection, speciation can occur under a broader range of parameters (yellow area). Below the yellow contour, speciation occurred within 10^5 generations in more than 50% of the simulations. **(B and C)** Orthogonal transects through parameter space illustrate a sharp increase of the time to speciation (green lines, in the absence of sexual selection) above a certain rate of migration [(B), $m > 0.3$] and below a critical strength of selection on the ecological character [(C), $\sigma > 0.75$]. With sexual selection, the time to speciation (yellow lines) increases gradually: As m and σ increase and ecological disruptive selection becomes weaker, higher values of the mating characters (red, female choosiness; blue, male investment into ornament expression) are required for the population to split. Points are mean \pm SD (denoted by error bars) over 20 replicate simulations; other parameters are the same as those in Fig. 2.



intensity of stabilizing selection within habitats (σ). Reduced migration between habitats (lower m) and increased selection for ecological specialization (lower σ) both result in stronger disruptive ecological selection across habitats on the population as a whole (see SOM). Without the help of sexual selection, extreme combinations of parameters are required to induce speciation (Fig. 3, green area), but when mate choice based on local adaptation is added, the constraints on ecological parameters are considerably relaxed. Even relatively weak ecological disruptive selection can be intensified by sexual selection up to the level that is required for ecological speciation (Fig. 3, yellow area).

Natural and sexual selection are often depicted as opposing forces, but they can work in concert (25, 26). Our model highlights how natural and sexual selection reinforce each other's actions in the context of adaptive speciation. Spatially heterogeneous selection pressures and migration between habitats can support the accumulation of genetic variation in ecological characters. Females enjoy durable benefits from choosing locally adapted males, unlike in populations that experience directional selection, where fitness variation is quickly depleted once sexual selection becomes stronger (16, 25–27, 29, 30). Once a mating preference for locally adapted partners has evolved, sexual selection reinforces assortative mating and lowers the fitness of hybrids. This twofold effect of mate choice on pre- and post-zygotic reproductive isolation is likely to extend to cases where mate choice depends on direct benefits. Disruptive ecological selection could also be amplified by intrasexual selection, if local adaptation interferes with displays of

condition that are used as signals in contests. Sexual selection would not be quite as effective in facilitating speciation in that case, however, because intrasexual selection does not necessarily strengthen assortative mating.

Our model differs from existing models of speciation by sexual selection in that it does not require the divergence of mating preferences between incipient species. Instead, gene flow between species is suppressed as a result of the genotype-by-environment interaction that determines mate attractiveness. Speciation on the basis of divergence of preferences and mating signals is fraught with complications due to the difficulty of supporting stable variation in mating preferences and maintaining linkage disequilibrium between mating traits and ecological characters (3–6, 9, 10, 13, 14); these issues are irrelevant to our model. In our model, incipient species only differ in ecological characters and not with respect to sexual preferences or ornamentation. Accordingly, speciation is more cryptic than in traditional models of speciation by sexual selection, at least during and shortly after the speciation process. In the long run, one would expect cryptic reproductively isolated habitat specialists to accumulate noticeable differences and species recognition traits.

Our results address a long-standing critique of models of sympatric speciation (3, 4, 6), which often rely on magic traits (5) to link ecological performance and assortative mating. In the case of host race specialization and comparable ecological contexts, it is widely recognized that ecology and mate choice are intimately intertwined. However, by lack of a general explanation for their presence, strong associations

between ecological specializations and mating behavior are often dismissed as unlikely. Sexual selection acting on indicators of local adaptation could provide such a general explanation, as is increasingly being illustrated by empirical studies examining mate choice in its ecological context [(10) and references therein]. Local adaptation and the maintenance of ecologically relevant variation are ubiquitous in natural populations. Such variation can serve as raw material for adaptive mate choice whenever sexual ornaments reflect performance under the local ecological conditions. Sexual selection and disruptive ecological selection can then reinforce each other, eventually leading to ecologically specialized and reproductively isolated sister species. Therefore, the scope for ecological speciation may not be limited by the presence of fortuitous pleiotropy between ecological and mating traits, but rather by the evolution of reliable signals of local adaptation from which such pleiotropy inevitably emerges.

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Figs. S1 to S3

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Structure of the LKB1-STRAD-MO25 Complex Reveals an Allosteric Mechanism of Kinase Activation

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The LKB1 tumor suppressor is a protein kinase that controls the activity of adenosine monophosphate-activated protein kinase (AMPK). LKB1 activity is regulated by the pseudokinase STRAD α and the scaffolding protein MO25 α through an unknown, phosphorylation-independent, mechanism. We describe the structure of the core heterotrimeric LKB1-STRAD α -MO25 α complex, revealing an unusual allosteric mechanism of LKB1 activation. STRAD α adopts a closed conformation typical of active protein kinases and binds LKB1 as a pseudosubstrate. STRAD α and MO25 α promote the active conformation of LKB1, which is stabilized by MO25 α interacting with the LKB1 activation loop. This previously undescribed mechanism of kinase activation may be relevant to understanding the evolution of other pseudokinases. The structure also reveals how mutations found in Peutz-Jeghers syndrome and in various sporadic cancers impair LKB1 function.

Loss-of-function mutations in the tumor suppressor LKB1 cause the rare inherited disease Peutz-Jeghers syndrome (PJS) in humans (1) and are associated with various sporadic cancers, in particular non-small cell lung cancer (2). One prominent function of LKB1 is to ensure that growth and division are coupled to the availability of cellular energy. LKB1 phosphorylates and activates the adenosine monophosphate-activated protein kinase (AMPK) when energy levels are low, thereby leading to inhibition of signaling pathways that promote proliferation (3). The therapeutic effects of AMPK-activating drugs (e.g., metformin) on tumor growth (4) or blood glucose levels (5) are dependent on activation of AMPK by LKB1. Another key role of LKB1 is to control cell polarity, which may be mediated by AMPK (6) or by a group of AMPK-related protein kinases, including microtubule affinity-regulating kinases (MARKs, homologous to the *Caenorhabditis elegans* kinase Par-1) (7)

that are also phosphorylated and activated by LKB1 (8).

In cells, LKB1 is found in a 1:1:1 heterotrimeric complex with the pseudokinase STRAD (Ste20-related adaptor) (9) and the scaffolding MO25 (mouse protein 25) (10). There are two closely related human isoforms of both STRAD (STRAD α and STRAD β) and MO25 (MO25 α and MO25 β) that similarly interact with LKB1 (11). Unlike the majority of protein kinases, which are regulated by phosphorylation, LKB1 is activated by binding to STRAD and MO25 (11, 12) through an unknown, phosphorylation-independent, molecular mechanism. Structural analysis of MO25 α reveals a helical-repeat, horseshoe-shaped protein that interacts with the C-terminal WEF (Trp-Glu-Phe) motif of STRAD α through a hydrophobic pocket located on its convex C-terminal surface (13). The structure of STRAD α complexed with MO25 α reveals additional interactions between the concave surface of MO25 α and the regulatory α C helix of STRAD α (14). STRAD α , despite being a catalytically inactive pseudokinase, adopts a closed conformation typical of fully active protein kinases. The closed conformation of STRAD α is maintained through its cooperative binding to adenosine triphosphate (ATP) and MO25 α . Mutations that inhibit binding to ATP and MO25 α

prevent LKB1 activation, which suggests that the active conformational state of STRAD α may be required for activation of LKB1 (14).

We report the crystal structure of the LKB1-STRAD α -MO25 α heterotrimeric complex. We used an insect cell expression system to produce an active core LKB1-STRAD α -MO25 α heterotrimeric complex, comprising the kinase domain of LKB1 (residues 43 to 347), complexed with the pseudokinase domain of STRAD α (residues 59 to 431) and full-length MO25 α (figs. S1 and S2). The crystal structure of the heterotrimeric complex with a catalytically inactive mutant of LKB1 (Asp¹⁹⁴ \rightarrow Ala, preventing Mg²⁺ ion binding but not assembly of the complex; fig. S2B) in complex with the ATP analog adenylyl-5'-yl imidodiphosphate (AMP-PNP) was solved and refined to 2.65 Å (table S1). There are two heterotrimeric complexes in the asymmetric unit displaying similar conformations (RMSD = 0.5 Å on 791 C α atoms). Both STRAD α and LKB1 are in complex with AMP-PNP, displaying binding modes typical of other protein kinases (fig. S3) (15).

The LKB1 heterotrimer has an overall compact globular shape with considerable interactions among all of the three subunits (Fig. 1A and fig. S4). The pseudokinase domain of STRAD α binds to the kinase domain of LKB1. The horseshoe-shaped MO25 α acts as a scaffold for assembly of the heterotrimer by binding both LKB1 and STRAD α through highly conserved residues on the concave face of its helical repeats (Fig. 1A and fig. S4B). MO25 α binds to STRAD α through a large (2930 Å²) interface centered on the regulatory helix α C of STRAD α (Fig. 1A). The structure of the STRAD α -MO25 α complex within the heterotrimer is similar to the binary STRAD α -MO25 α complex structure (14) (RMSD = 0.5 Å on 529 C α atoms; fig. S5), including ordered electron density for the STRAD α C-terminal WEF motif interacting with a pocket on MO25 α (13, 14). The remaining MO25 α concave surface is engaged in contacts (1580 Å²) with the LKB1 activation loop, helix α I, and the C terminus of helix α C (Fig. 1A and fig. S4). The interface between LKB1 and STRAD α mainly involves the C lobe of STRAD α and both N and C lobes of LKB1 (1840 Å²; Fig. 1C and fig. S4) and is comparable in size to the interaction between LKB1 and MO25 α .

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