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Science **318**, 95 (2007);
DOI: 10.1126/science.1141506

Sex Chromosome–Linked Species Recognition and Evolution of Reproductive Isolation in Flycatchers

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Interbreeding between species (hybridization) typically produces unfit offspring. Reduced hybridization should therefore be favored by natural selection. However, this is difficult to accomplish because hybridization also sets the stage for genetic recombination to dissociate species-specific traits from the preferences for them. Here we show that this association is maintained by physical linkage (on the same chromosome) in two hybridizing *Ficedula* flycatchers. By analyzing the mating patterns of female hybrids and cross-fostered offspring, we demonstrate that species recognition is inherited on the Z chromosome, which is also the known location of species-specific male plumage traits and genes causing low hybrid fitness. Limited recombination on the Z chromosome maintains associations of Z-linked genes despite hybridization, suggesting that the sex chromosomes may be a hotspot for adaptive speciation.

Reproductive isolation is traditionally viewed as an incidental by-product of genetic divergence during geographic isolation. However, many diverged populations come into contact before complete reproductive isolation has evolved. In such cases, natural selection against maladaptive interbreeding (hybridization) may complete speciation by reinforcing a tendency to mate with one's own kind (assortative mating) (*1*–*3*). This process, termed reinforcement, is of potentially great evolutionary significance because it suggests that reproductive isolation itself can be an adaptive response to natural selection. However, the empirical support for reinforcement is limited, and the conditions under which it can theoretically occur are sometimes strict (*2*, *4*).

Selection for assortative mating favors the buildup of genetic associations between components of assortative mating (species-specific traits and the preferences for these traits) and between such pre-zygotic barriers and low hybrid fitness (post-zygotic barriers). However, DNA recombination during hybridization breaks these associations necessary for speciation (*5*). Using a combination of field experiments, molecular techniques, and long-term breeding data from hybrid zones of wild birds in the Czech Republic and Sweden, we tested this central problem of reinforcement: How can the traits involved in reproductive isolation remain associated in the face of gene flow?

Two solutions to this problem have been suggested. First, species recognition can occur through a "one-allele mechanism": a single allele, established in both incipient species, can cause assortative mating (*2*, *5*, *6*). For example, sexual imprinting is a widespread phenomenon in birds (*7*), whereby females learn the traits of their fathers and later prefer similar males as mates. An allele causing sexual imprinting would make recombination irrelevant, because it would result in opposite mate preferences in the two species (*7*–*9*). Second, recombination can be suppressed through, for example, physical linkage of genes (*2*, *5*, *10*–*13*). Recent theoretical studies have highlighted the idea that sex linkage (placement on a sex chromosome) of species-recognition genes may enhance reinforcement when, as is often the case, genes causing low hybrid fitness are also sex-linked (*9*, *13*–*15*). We tested whether species recognition is due to sex-linked preferences, sexual imprinting, or autosomally inherited preferences in an avian system with evidence for reinforcement (*16*).

Where the breeding distributions of pied flycatchers [*Ficedula hypoleuca* (pied)] and collared flycatchers [*F. albicollis* (collared)] overlap (sympatry), the populations mate assortatively with respect to species (*17*). However, some interbreeding occurs [2 to 5% of pairs (*17*)], and the resulting hybrids have reduced fitness, female hybrids being sterile (*17*). Despite gene flow through male hybrids (*18*), both male plumage and female mate preferences have diverged furthest in sympatric areas, presumably to facilitate avoiding hybridization (*16*).

Genes causing hybrid incompatibility and genes influencing the expression of diverged male plumage traits are both located on the Z sex chromosome in flycatchers (*18*). Genes on the Z chromosome are in general likely to recombine less than if they were on autosomes; for instance, because sex chromosome recombination can occur only in the homogametic sex (supporting online text). In fact, previous studies have failed to detect any interspecific recombination between the Z chromosomes of hybridizing flycatchers, whereas autosomes recombine between the species (*18*). If genes on the Z also determine species recognition by females, recombination between loci that are important for reinforcement would be greatly reduced.

In birds, females inherit half of their autosomal genes from either parent, but their single Z chromosome solely from their father. We used this fact to distinguish between whether species recognition is inherited on autosomes or is paternally determined (by Z linkage or learned by sexual imprinting on fathers). If mate preferences are paternally determined, the mate choice of female hybrids should vary according to the species of their father, corresponding to that of pure females of the paternal species. To determine the paternal and maternal species of female hybrids, as well as their status as F_1 hybrids, we used species-specific genetic markers at the Z chromosome (paternally inherited) and in the mitochondrial genome (maternally inherited) (*19*).

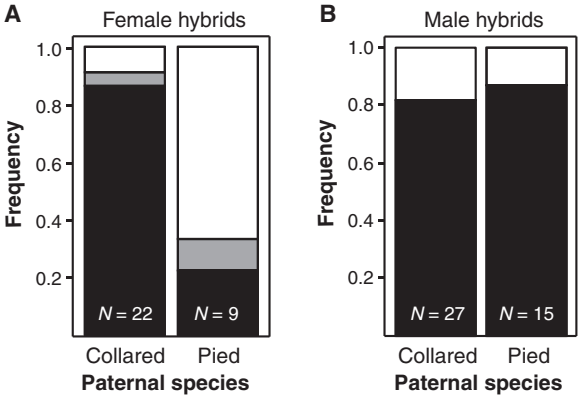

Fig. 1. Mating patterns of hybrid flycatchers. Female hybrids (**A**) predominately mated with males belonging to the same species as their father regardless of whether the father was a collared flycatcher or a pied flycatcher (black, collared partner; white, pied partner; gray, hybrid partner). In contrast, in male hybrids (**B**) no relationship was present between paternal species and the species of their mate.

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We found that female hybrids having a pied father predominately mated with a pied male, whereas female hybrids having a collared father predominately mated with a collared male (Fig. 1A). Only 4 out of 31 female hybrids mated with a male of the maternal species. Thus, there was a nonrandom association between the species of a female hybrid's father and the species of her mate [$x_2^2 = 12.37$, exact $P = 0.001$, $N = 31$ hybrids; excluding matings with male hybrids, Fisher's exact test, $P = 0.001$, $N = 29$]. This pattern was present in both Czech ($P = 0.048$, $N = 9$) and Swedish ($P = 0.062$, $N = 22$) sympatric areas.

Male hybrids, on the other hand, inherit a Z chromosome from both parental species and are unaffected by sexual imprinting because male flycatchers do not discriminate against heterospecific partners (*20*). As expected, there was no association between the paternal species of male hybrids and the species of their mate (Fisher's exact test, $N = 42$, $P = 1$, Fig. 1B). This implies that the pattern observed in female hybrids is not simply some artefact of hybrids but is consistent only with paternal inheritance (through Z linkage or sexual imprinting) of species recognition; this therefore eliminates autosomal inheritance as the mechanism behind species-assortative mating.

Flycatchers provide an ideal system in which to disentangle sexual imprinting from Z linkage, because hybridizing females often engage in extrapair copulations with conspecific males (*17*), resulting in purebred offspring being reared by cuckolded heterospecific males. We used multigenerational breeding data to analyze the mate choices of such pure female flycatchers that had been reared by a male of the other species. If assortative mating is due to sexual imprinting, these females should prefer heterospecific males as their mates. We also experimentally cross-fostered offspring between nests of the two species and recorded the mate choice of cross-fostered females that returned to breed. Under genetic inheritance of species recognition, cross-fostered females should mate as other females of their own species, whereas under sexual imprinting, they should mate as females of the foster father's species (*19*).

In contrast to prevailing views on the development of sexual preferences in birds (*7*), Fig. 2 shows that females did not become sexually imprinted on their social father. Instead, females with a heterospecific foster father mated with conspecific males to the same extent as other females of their own species did. This conclusion applies to females of both species, using both naturally (Fig. 2, A and B) and experimentally (Fig. 2, C and D) cross-fostered offspring and regardless of whether expected mating patterns were calculated from all breeding pairs or from recruits of known parents only. Although some of the sample sizes are small because of the rarity of these events, the evidence is overwhelming that species recognition does not develop by sexual imprinting in these birds. Instead, our results imply that species-assortative mating has a genetic basis.

Assuming Mendelian inheritance, the different mate preference of the two kinds of female hybrids is solely consistent with Z linkage. A non-Mendelian epigenetic possibility is that the preference genes are autosomally inherited, but only the paternal allele is expressed (maternal genomic imprinting). However, such parent-of-origin effects have not been found in birds (*21*).

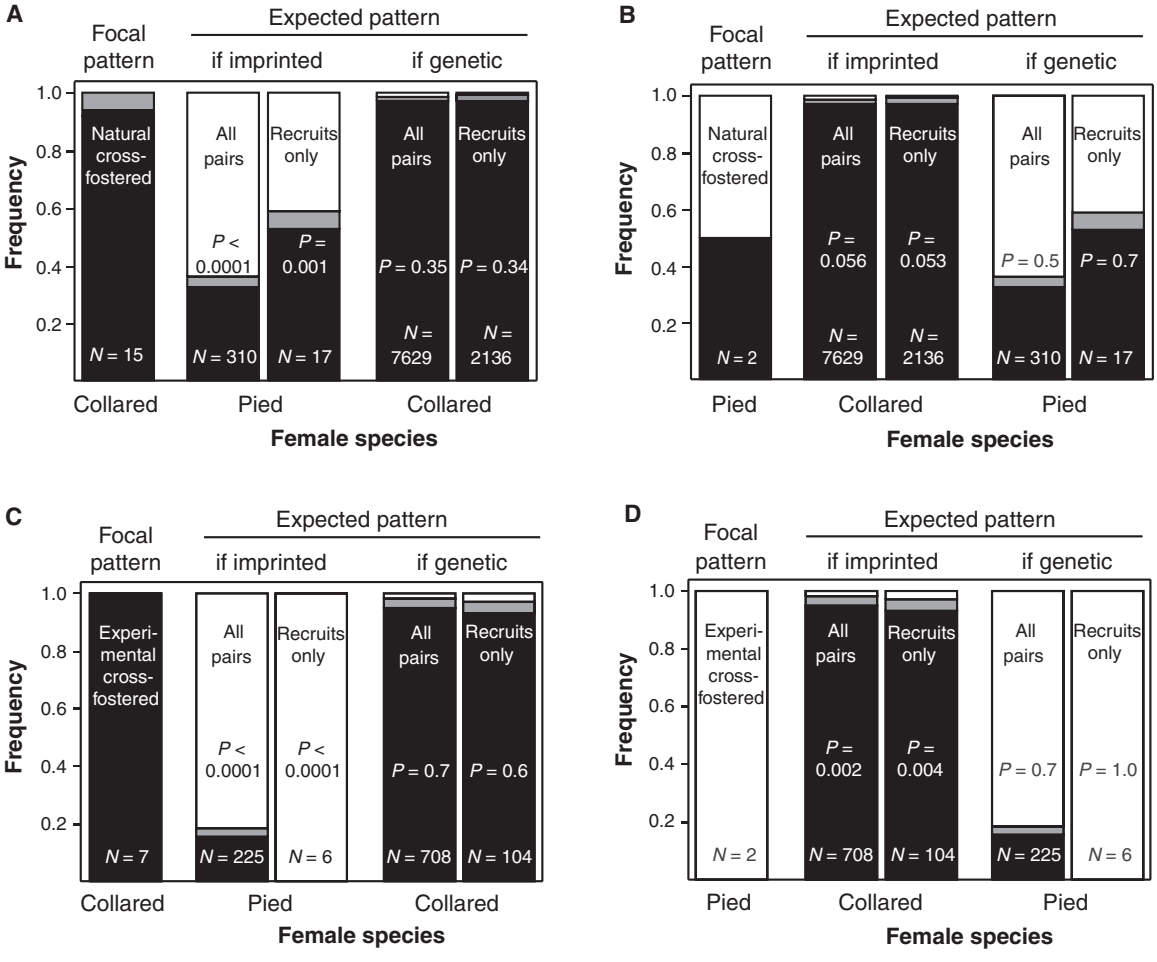

Fig. 2. Mating patterns of female flycatchers reared by heterospecific foster fathers compared to expectations based on sexual imprinting or genetic inheritance of species recognition. In each panel, the first bar shows the mating pattern of females reared by heterospecific foster fathers (focal pattern; black, collared flycatcher partner; white, pied flycatcher partner; gray, hybrid partner). The upper panels (**A** and **B**) show mating patterns of females naturally raised in mixed-species nests by cuckolded heterospecific stepfathers (natural cross-fostered), and the lower panels (**C** and **D**) show mating patterns of females experimentally transferred to heterospecific nests (experimental cross-fostered). The left panels [(A) and (C)] refer to collared flycatchers, whereas the right panels [(B) and (D)] present pied flycatchers. The expected mating patterns (specific to the population in which the focal patterns were obtained) were constructed in two ways, using either the mating pattern in the population (all pairs) or using only the mating pattern of females born in nests of known pure pairs (recruits only). *P* values indicate the exact binomial two-tailed probability of obtaining the observed focal mating pattern of females reared by heterospecific fathers (number of such females mating with conspecific males versus other males) under the different expected proportions. *N* indicates number of breeding pairs.

Moreover, they are not expected to occur in birds according to one predominant theory of the evolution of genomic imprinting, and genes that are imprinted in mammals show ordinary bi-allelic expression in birds (*21*). We therefore conclude that species-assortative mating preferences in flycatcher hybrid zones are mainly due to Z-linked genes.

All three major components of reproductive isolation (species recognition, species-specific male traits, and hybrid incompatibilities) being Z linked in flycatchers should facilitate an evolutionary response to natural selection against hybridization. This is because genetic associations between the male and the female components of pre-zygotic barriers to gene flow, as well as between pre-zygotic and post-zygotic barriers, can easily be maintained (see supporting online text for further discussion of the flycatcher system). Our results suggest that some organisms may be prone to speciation through reinforcement because of the mediating role of the sex chromosomes. Compared to autosomally inherited species recognition, both sex linkage and sexual imprinting may allow incipient species to avoid a collapse in assortative mating during secondary contact and be less likely to succumb to gene flow and fusion (*9*). However, paternal sexual imprinting requires that females be socially exposed to their father, which is not always true even in birds. Conversely, because reduced hybrid fitness is commonly caused by sex-linked incompatibilities (*3*), sex linkage of species recognition might provide a general connection between key components of reproductive isolation, which facilitates adaptive speciation in the face of gene flow.

Sex-chromosome linkage of species-assortative female mate preferences may be widespread, but few previous studies have explicitly investigated the mechanism of species recognition in hybrid zones. Even fewer studies have provided additional information on the genetics of hybrid fitness and the preferred traits, or evidence for reinforcement (*22*–*25*). Nevertheless, disproportionately many genes involved in reproductive isolation seem to be located on the sex chromosomes (*15*, *26*, *27*). In Lepidoptera, which also have heterogametic females, sex-linked traits seem to be more associated with reproductive isolation than in other insects (*28*), and it has been suggested that ornaments and preferences for these ornaments evolve more readily in organisms with ZW than with XY sex chromosomes (*26*, *29*). Although speciation would benefit from any kind of linkage (or other recombination-suppressing mechanism) that can maintain these genetic associations, traits involved in pre-zygotic isolation may simply be more likely to occur on sex chromosomes than on autosomes and possibly more likely on Z than on X chromosomes (*27*). Sex chromosomes in general, and the Z in particular, may therefore be hotspots for speciation genes.

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30. We thank T. F. Hansen, Ø. H. Holen, A. J. van Noordwijk, K. van Oers, F. Pulido, T. O. Svennungsen, and M. Visser for suggestions. The study was supported by grants from the Swedish Research Council, the Research Council of Norway, Formas, the Netherlands Organization for Scientific Research, NSF, the Czech Ministry of Education, and the Czech Science Foundation.

Supporting Online Material

www.sciencemag.org/cgi/content/full/318/5847/95/DC1
Materials and Methods
SOM Text
References

20 February 2007; accepted 15 August 2007
10.1126/science.1141506

Microbial Population Structures in the Deep Marine Biosphere

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The analytical power of environmental DNA sequences for modeling microbial ecosystems depends on accurate assessments of population structure, including diversity (richness) and relative abundance (evenness). We investigated both aspects of population structure for microbial communities at two neighboring hydrothermal vents by examining the sequences of more than 900,000 microbial small-subunit ribosomal RNA amplicons. The two vent communities have different population structures that reflect local geochemical regimes. Descriptions of archaeal diversity were nearly exhaustive, but despite collecting an unparalleled number of sequences, statistical analyses indicated additional bacterial diversity at every taxonomic level. We predict that hundreds of thousands of sequences will be necessary to capture the vast diversity of microbial communities, and that different patterns of evenness for both high- and low-abundance taxa may be important in defining microbial ecosystem dynamics.

The interrogation of DNA from environmental samples has revealed new dimensions in microbial diversity and community-wide metabolic potential. The first analysis of a dozen polymerase chain reaction (PCR) amplicons of ribosomal RNA (rRNA) sequence from a mixed bacterioplankton population revealed the ubiquitous SAR11 cluster (*1*), and a recent environmental shotgun sequence survey of microbial communities in the surface ocean has identified 6.1 million predicted proteins (*2*, *3*). To realize the full potential of metagenomics for modeling energy and carbon flow, microbial biogeography, and the relationship between microbial diversity and ecosystem function, it is necessary to estimate both the richness and evenness of microbial population structures.

We used a tag sequencing strategy that combines the use of amplicons of the V6 hypervariable region of small-subunit (SSU) rRNA as proxies for the presence of individual phylotypes [operational taxonomic units (OTUs)] with massively parallel sequencing. Our goal was to provide assessments of microbial diversity, evenness, and community structure at a resolution two to three orders of magnitude greater than that afforded by cloning and capillary sequencing of longer SSU rRNA amplicons (*4*). We used this strategy to attempt an exhaustive characterization of the bacterial and archaeal diversity at two