

Sympatric speciation by allochrony in a seabird

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The importance of sympatric speciation (the evolution of reproductive isolation between codistributed populations) in generating biodiversity is highly controversial. Whereas potential examples of sympatric speciation exist for plants, insects, and fishes, most theoretical models suggest that it requires conditions that are probably not common in nature, and only two possible cases have been described for tetrapods. One mechanism by which it could occur is through allochronic isolation—separation of populations by breeding time. *Oceanodroma castro* (the Madeiran or band-rumped storm-petrel) is a small seabird that nests on tropical and subtropical islands throughout the Atlantic and Pacific Oceans. In at least five archipelagos, different individuals breed on the same islands in different seasons. We compared variation in five microsatellite loci and the mitochondrial control region among 562 *O. castro* from throughout the species' range. We found that sympatric seasonal populations differ genetically within all five archipelagos and have ceased to exchange genes in two. Population and gene trees all indicate that seasonal populations within four of the archipelagos are more closely related to each other than to populations from the same season from other archipelagos; divergence of the fifth sympatric pair is too ancient for reliable inference. Thus, seasonal populations appear to have arisen sympatrically at least four times. This is the first evidence for sympatric speciation by allochrony in a tetrapod, and adds to growing indications that population differentiation and speciation can occur without geographic barriers to gene flow.

Oceanodroma castro | phylogeography | genetic isolation | seasonal populations | storm-petrel

Speciation—the evolution of reproductive isolation between populations—is thought generally to involve the gradual accumulation of genetic differences between geographically isolated (allopatric) populations through selection or genetic drift (reviewed in ref. 1). Although this “allopatric” model of speciation is widely accepted, it does not provide a satisfactory mechanism for the origin of many species, such as sympatric sister species, and several alternatives have been proposed (reviewed in ref. 1). According to various “sympatric” models, reproductive isolation could evolve in the absence of geographic isolation through polyploidization (e.g., in flowering plants) or nonrandom mating [e.g., according to host in phytophagous insects or brood parasitic birds, migratory route in birds, or breeding time (allochrony) in salmon and periodical cicadas] (1–4). Sympatric speciation was first proposed by Darwin but was refuted by Mayr and has been controversial ever since (reviewed in refs. 1 and 3). Although sympatric speciation is possible in theory, most models require conditions that are probably uncommon in nature, such as an appropriate balance between selection and recombination, linkage between genes involved in ecological specialization and reproductive isolation, and/or a small number of loci controlling local adaptation, and habitat and mate preference. It also requires populations to be able to coexist ecologically during and after the evolution of reproductive isolation (1, 3, 5, 6).

Sympatric speciation is difficult to demonstrate in nature. Potential examples exist for plants, insects, and fishes (1–7), but

only two possible cases have been described for tetrapods: host-specific races of brood parasitic indigobirds (*Vidua* spp.) (8, 9) and ecologically segregated races of *Nesospiza* buntings on Tristan da Cunha (10). Coyne and Orr (ref. 1, p. 142) delineate four criteria that must be met to provide a convincing case: “1. The species must be largely or completely sympatric. 2. The species must have substantial reproductive isolation, preferably based on genetic differences. 3. The sympatric taxa must be sister groups. 4. The biogeographic and evolutionary history of the groups must make the existence of an allopatric phase very unlikely.” Unfortunately, the geographic and phylogenetic signatures of sympatric speciation are easily obscured by range expansion, extinction, and lineage sorting (1, 11). However, modern methods of molecular analysis are enabling increasingly rigorous tests of speciation models.

Oceanodroma castro (the Madeiran or band-rumped storm-petrel; Procellariiformes: Hydrobatidae) provides a useful test model for sympatric speciation. This small pelagic seabird forages throughout tropical and subtropical regions of the Atlantic and Pacific Oceans and breeds on several islets in Japan, Hawaii, the Galapagos, and the northeast and central Atlantic (12) (Fig. 1). The timing of breeding varies considerably among colonies [supporting information (SI) Table 2]: at one extreme, some populations have a single, compact breeding season (e.g., Japan); at other islands, the nesting season is much more protracted (e.g., Cape Verde); at the other extreme, several colonies have two distinct laying periods separated by an interval with no breeding (specifically, the Azores, Desertas, and the Galapagos). Evidence from breeding and molting phenology, feather mercury concentrations, band (ring) returns, morphology, vocalizations, and mtDNA sequences suggests that different populations nest in the same areas—even in the same burrows—at some of these colonies (12–20). Differences in all these characters between seasonal populations within the Azores indicate that they represent reproductively isolated species (16, 18, 20) and thus meet the first of Coyne and Orr's criteria for sympatric speciation (1). Seasonal populations of *O. castro* may therefore represent cases of sympatric divergence and speciation through habitat preference, specifically, breeding season (allochrony). Here, we compare variation in five microsatellite loci and a portion of the mitochondrial control region among 17 populations of *O. castro*, including four known pairs of sympatric seasonal populations and samples from two seasons from Cape

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ably reproductively isolated, and virtually completely segregated on the mtDNA gene tree (Fig. 4); given that they are diagnosable by season as well as by morphology and vocalizations (16, 18), they constitute species by most definitions (1). Sympatric seasonal populations within Cape Verde appear to be genetically isolated; however, they are not yet reciprocally monophyletic on the gene tree or diagnosable by any character other than breeding season, so represent an earlier stage of speciation than the Azores populations. Sympatric seasonal populations in Desertas, Selvagem, and the Galapagos exhibit some genetic differentiation as well as slight morphometric differences (15, 17, 19) but are not genetically isolated.

In contrast, little differentiation exists either among cool season populations from different colonies within the northeast Atlantic, or between the two Azores hot-season colonies: Few estimates of population differentiation differ from zero (SI Table 5), and several estimates of migration and gene flow are significant (SI Tables 3 and 4).

Sympatric taxa are sister groups. Two lines of evidence indicate that sympatric seasonal populations of *O. castro* are sister taxa. First, within the mtDNA gene tree, haplotypes from Galapagos seasonal populations occur together within a strongly supported monophyletic clade (Fig. 4). Most haplotypes from Cape Verde seasonal samples also group together within a strongly supported monophyletic lineage. Haplotypes from Azores seasonal populations occur together within a strongly supported but poorly resolved Atlantic clade, although this clade also includes haplotypes from other northeast Atlantic colonies; the Azores hot-season population is approaching monophyly within this lineage. Haplotypes from the Desertas and Selvagem seasonal populations also occur together within the northeast Atlantic clade.

Second, on both the nuclear and mtDNA population trees, Galapagos seasonal populations form a monophyletic group, as do Cape Verde seasonal samples (Fig. 5); each of these lineages has strong support on the nuclear tree (Fig. 5A). Similarly, Azores seasonal populations occur within an unresolved but monophyletic clade that includes other northeast Atlantic cool-season populations; although they do not group together, note that a sister-taxon relationship would be quickly lost from the population tree if gene flow is ongoing among northeast Atlantic cool-season populations.

An historical allopatric phase is very unlikely. If seasonal populations arose allopatrically, or arose only once sympatrically, evidence of historical fragmentation and secondary contact should be present in the form of deep branches in the gene tree (11, 22). This historical fragmentation should be apparent whether or not the original colonies still exist. No evidence was found for historical fragmentation or secondary contact between any sympatric seasonal populations either from the general shape of the gene tree (Fig. 4) or from nested clade analysis of the northeast Atlantic populations (20) or nested contingency analysis of the Galapagos clade (19).

Thus, seasonal populations of *O. castro* in the Azores and Cape Verde meet all of Coyne and Orr's criteria for sympatric speciation (1). Seasonal populations in Desertas, Selvagem and the Galapagos also fulfill three of the criteria, but are probably not yet reproductively isolated.

At least two alternatives to sympatric origins are possible for seasonal populations of *O. castro*. One is that seasonal populations arose allopatrically, followed by sympatry and hybridization. This could result in the observed clustering of sympatric seasonal populations on the population trees. The possibility of hybridization is supported by 10 control-region haplotypes that are shared between seasonal populations (SI Table 6) and by polyphyly within the mtDNA gene tree (Fig. 4). However, three observations argue against this scenario. First, hybridization would have to be extensive to result in the observed population

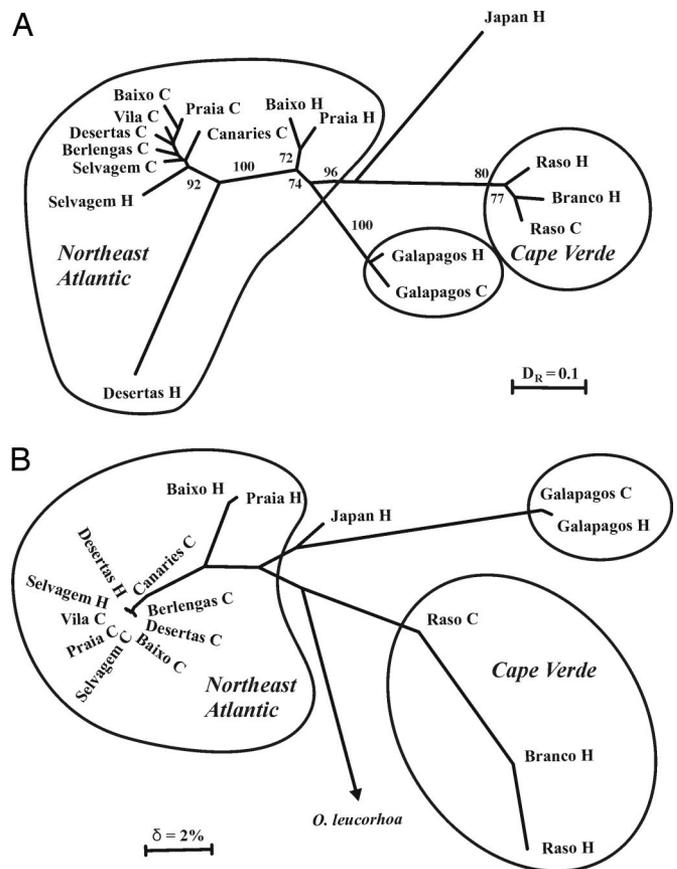


Fig. 5. Unrooted neighbor-joining trees for *O. castro* populations based on microsatellite variation (A), and mitochondrial control region sequences (B). Numbers on branches for A are support from bootstrapping across individuals (values <70% not shown). *O. leucorhoa*, Leach's storm-petrel. C, populations breeding in the Northern Hemisphere cool season; H, populations breeding in the Northern Hemisphere hot season.

and gene trees, but results from BayesAss and MDIV indicate that there is no exchange of either nuclear or mitochondrial genes between seasonal populations within the Azores or Cape Verde (SI Tables 3 and 4). Second, as noted above, no evidence of historical fragmentation and/or secondary contact (hybridization) was found within the gene tree (Fig. 4). Finally, of the 10 haplotypes that are shared between sympatric seasonal populations (SI Table 6), most (eight) are located at or near the root of the gene tree (19, 20), indicating that they represent retained ancestral variation; only one haplotype each in the Galapagos and northeast Atlantic occurred on a branch tip, suggestive of possible contemporary gene flow or hybridization.

Another possible explanation is that seasonal populations originated on different islands within an archipelago (i.e., through microallopatry), followed by sympatry through range expansion (11). Although possible, this explanation is less parsimonious than sympatric origins, i.e., it requires more events: speciation plus range expansion. Furthermore, it must have happened multiple times to generate the population trees seen in Fig. 5. However, the possibility of microallopatric origins is difficult to preclude for the Azores seasonal populations: given that the Azores cool-season breeders appear to be part of a large, interbreeding metapopulation that includes colonies from the coast of mainland Portugal, Desertas, Selvagem, and the Canaries, cool-season breeders could have originated anywhere within the northeast Atlantic and expanded into the Azores after the origin of the Azores hot-season population. As noted previously,

samples were obtained by using primers OcL61 (20) and H530 (V.L.F. and P. Gulavita, unpublished data) by using the protocols of Smith *et al.* (20). Sequences were also obtained for two Leach's storm-petrels (*Oceanodroma leucorhoa*) to root the mtDNA trees. Nonoverlapping sequences were trimmed from the combined data set, generating 205 haplotypes (SI Table 6).

Genetic differentiation among populations was indexed by using pair-wise estimates of F_{ST} (microsatellites) and Φ_{ST} and δ (control region sequences using Kimura's two-parameter correction for multiple substitutions with $\gamma = 0.45$), with significance determined by 10,000 bootstrap replications using the program Arlequin. Estimation of contemporary gene flow from F_{ST} or its analogues involves several dubious assumptions, most notably, that populations are in equilibrium between migration and genetic drift (33). We therefore used molecular assignments to estimate gene flow from microsatellite variation by using the program BayesAss (34). Three million MCMC iterations were run, with a burn-in of 1,000,000 iterations and a sampling frequency of 2,000. Δ (the maximum amount a parameter can change each iteration) was set to 0.15 (the default value). Samples from the Galapagos and the Atlantic were analyzed separately because these populations form highly distinct lineages on the population trees; Japan was not included because it is highly divergent from the other populations and lacks seasonal populations. Female-mediated gene flow between population pairs (M in females per generation) was estimated from control region sequences by using a maximum-likelihood approach based on coalescent theory, by using the program MDIV (35); T_{MAX} and M_{MAX} were adjusted for each comparison to ensure that likelihood estimates equilibrated. Samples from the Galapagos, Cape Verde, and the northeast Atlantic were analyzed separately, and Japan was not included, as above. Canaries samples also were excluded because of small sample size. Estimates of M were tested for difference from 1 by using likelihood ratio tests.

MDIV also was used to test whether populations are genetically isolated. Specifically, likelihood ratio tests were used to determine whether divergence time (T , in N_f generations, where N_f is female effective population size) was >0 . Divergence time in years (t) was estimated as $T\Theta/2\mu$, where $\Theta = 2N_f\mu$, and μ is the per-fragment mutation rate [estimated at 3.3×10^{-5} , assuming a divergence rate of

21% per million years for Domain I of the mitochondrial control region of birds (36)].

To visualize the genetic relationships among populations, a phenetic population tree was generated from microsatellite data by neighbor-joining on Roger's genetic distances by using the program Populations (version 1.2.3beta, www.bioinformatics.org/project/?group_id=84); confidence for branches was determined by bootstrapping across individuals. Other distance measures [Nei's minimum genetic distance (which increases linearly with time), Wright's linearized F_{ST} , and Reynold's unweighted distance] gave similar results. A phenetic population tree was generated from control-region sequences by neighbor-joining on Kimura-two-parameter corrected distances (δ) between population samples by using Mega (version 3.1) (37). A gene tree was generated for the control region haplotypes by using statistical parsimony, but several branch lengths exceeded the connection limit so that relationships among key populations could not be determined (20). Therefore, a neighbor-joining tree was generated for Kimura-two-parameter corrected distances between haplotypes by using Mega; use of other tree-construction algorithms or distance measures made only minor changes to branch tips. Support for branches was determined by interior branch tests (38).

A rejection level (α) of 0.05 was used throughout, with sequential Bonferroni corrections applied to minimize Type I errors.

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