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Divergent selection on bill morphology contributes to nonrandom mating between swamp sparrow subspecies

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Traits subject to ecologically based divergent selection that also affect nonrandom mating (i.e. 'magic traits') may hasten incipient speciation. In this study, we investigated whether nonrandom mating results from ecological divergence between swamp sparrow, *Melospiza georgiana*, subspecies. As a by-product of adaptive divergence in bill morphology between populations of swamp sparrows, there is a corresponding divergence in features of song shown to be salient to females, potentially providing a mechanism of reproductive isolation. Thus, female mating preferences for song could result in prezygotic isolation that prevents gene flow between subspecies. In this study we tested the hypothesis that female preferences for song provide a mechanism of reproductive isolation that limits gene flow between subspecies. Using copulation solicitation assays, we found that female coastal plain swamp sparrows showed a significant preference for consubspecific songs over heterosubspecific songs. We further tested whether mating preferences in coastal populations explain observed differences in song between subspecies. We found evidence that mating preferences in coastal females may explain the evolution of some acoustic features of song. We suggest that the bill may be an example of a 'magic trait' that contributes to continued divergence of swamp sparrow subspecies.

Understanding the processes that lead to geographical variation within species can help resolve questions about the origin of species (Mayr 1942; Dobzhansky 1951; Orr & Smith 1998; Schluter 2000; Coyne & Orr 2004). However, the evolutionary processes involved in progressing from geographical variation within species to the formation of new species are rarely observed in action (Schluter 2001). Part of the difficulty lies in identifying independently evolving lineages among morphologically divergent populations, because recently separated populations often lack the molecular hallmark of evolution: divergence in neutral genetic variation (reviewed in: Funk & Omland 2003; Zink 2004). Discordance between genotypic and phenotypic divergence provides an opportunity to understand the workings of natural selection and the early processes of speciation (Mayr 1963). For example, ecological speciation is driven by distinct local environmental conditions, resulting in divergent natural selection strong enough to shape adaptive morphological traits, even when the ecological divergence has been recent or gene flow is still strong (Neigel & Avise 1986; Orr & Smith 1998; Rundle & Nosil 2005). If divergent selection acts on a trait that influences both ecological adaptation and reproductive isolation (i.e. 'magic trait'), then ecologically based divergence may lead to speciation even in the presence of gene flow (de León et al. 2010; Servedio et al. 2011). Despite the intriguing possibility that ecological speciation might explain a great deal of the variety present in nature, evidence for this evolutionary mechanism remains sparse, and more tests from wild populations are needed (Schluter 2001).

Tidal marshes are a potential source of ecological speciation because they provide sharp environmental discontinuities from inland marshes (Adam 2000). Conditions unique to tidal marsh habitat, such as tidal flooding and high salinity, favour a specialized biotic community (Malamud-Roam et al. 2006). Vertebrates that occupy tidal marshes show parallel evolution of traits, such as darker coloration and larger bills in birds (Greenberg & Maldonado 2006), suggesting an adaptive response to the unique conditions present in tidal marshes (Endler 1986; Grenier & Greenberg 2005). This divergent community provides an opportunity to investigate a mechanism of ecological speciation in a system (tidal marshes) and focal species (the swamp sparrow, *Melospiza georgiana*) for which good *prima facie* evidence exists for ongoing adaptive divergence (Grenier & Greenberg 2005; Ballentine & Greenberg 2010). Swamp sparrows are widely distributed in North American wetlands, but coastal plain swamp sparrows, *Melospiza georgiana nigrescens*, are recent colonists

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of tidal marshes (ca. 12 000 years ago) and are morphologically divergent from inland subspecies, *Melospiza georgiana georgiana* (Greenberg & Droege 1990; Greenberg et al. 1998; Grenier & Greenberg 2005; Greenberg & Maldonado 2006). Despite lack of divergence in neutral genetic variation in swamp sparrow subspecies (Balaban 1988; Greenberg et al. 1998), results of a recent common garden experiment suggest that morphological divergence has an underlying genetic basis (Ballentine & Greenberg 2010) that is likely the result of selection (Grenier & Greenberg 2005).

Rapid divergence of swamp sparrow subspecies with strong selection is consistent with incipient ecological speciation. But a key prediction of ecological speciation in swamp sparrows, the rapid evolution of reproductive-isolating mechanisms (Schluter 2001), remains untested. Ecological speciation is especially favoured when a strong environmental gradient is coupled with divergence in traits important in reproductive isolation (Smith et al. 1997; Orr & Smith 1998; Slabbekoorn & Smith 2002; Smith & Benkman 2007). In coastal plain swamp sparrows, an increase in bill size by 10–15% has likely evolved in response to the divergent ecology of tidal marsh habitat (Greenberg & Droege 1990; Grenier & Greenberg 2005; Greenberg & Maldonado 2006). Thermal conditions in coastal marshes may favour larger bill size as a means to radiate heat in the more warm and humid conditions found in coastal marshes (Greenberg et al. 2012). Bird bills are a promising trait to investigate as a potential 'magic trait' because bill morphology is often a primary target of natural selection (Boag & Grant 1981; Grant & Grant 2002; Patten et al. 2004; Grant et al. 2006) and plays an important role in song production (Slabbekoorn & Smith 2002; Podos & Nowicki 2004) that may influence reproductive isolation. In this case, acoustic as well as morphological divergence has been faster than divergence in neutral genetic loci, implicating ecological selection, sexual selection, or a combination of both (Wilkins et al. 2013). Swamp sparrows provide an opportunity to test the possibility that bird bills may be an example of a magic trait in a case where ecological selection influences sexual selection. Variation in bill morphology influences divergence in song between swamp sparrow subspecies (Ballentine 2006; Liu et al. 2008). Because of the role of the bill in birdsong production and the importance of birdsong in mate choice, divergent ecological selection on bills may result in nonrandom mating between swamp sparrow subspecies.

The primary goal of this study was to test whether song can function as an isolating mechanism between subspecies of swamp sparrows by using an experimental approach. We focused on assaying mating preferences for consubspecific song in coastal females for two reasons: (1) gene flow is more likely to prevent divergence in small coastal populations and (2) female inland swamp sparrows discriminate against songs produced by coastal males, because females from inland populations prefer songs that are physically challenging (Ballentine et al. 2004). Coastal males are unable to produce songs that meet the level of performance preferred by inland females (Ballentine et al. 2004; Ballentine 2006) because large bills constrain song production (Podos 2001; Podos et al. 2004b). Therefore, we assumed that reproductive isolation would be maintained by female mating preferences for song in inland populations. However, nothing is known about female mating preferences for song in coastal populations. If coastal and inland females have similar song preferences, then females in coastal populations may not discriminate against males from inland populations, thus preventing divergence between populations. Alternatively, divergence in song between populations may have a significant influence on the ability of coastal females to recognize inland males as conspecifics (Price 1998). If females from coastal subspecies discriminate against songs of inland subspecies, then this would demonstrate that ecologically based selection on bills can result in reproductive isolation between subspecies of swamp sparrows, providing an example of a magic trait.

Geographical variation in bill morphology between subspecies provides an explanation for observed differences in vocal performance between swamp sparrow subspecies (Ballentine 2006). However, there are other consistent patterns of variation in song between swamp sparrow subspecies that are less linked to morphological variation (Ballentine 2006). For example: (1) coastal males sing with significantly increased complexity both within and between songs when compared to males of inland populations (Ballentine 2006; Liu et al. 2008) and (2) despite the lower vocal performance of coastal males, their songs have significantly faster trill rates than do the songs of inland males (Ballentine 2006; Liu et al. 2008). These observations provide important insight to formulate specific hypotheses about the role of female mating preferences in promoting differences in song between subspecies. If female mating preferences are driving the differences between subspecies in these acoustic features, then we predicted that coastal females would (1) prefer more complex songs and (2) prefer songs with faster trill rates. The secondary goal of this study was to test whether coastal females attend to features of song associated with observed differences in song between subspecies.

METHODS

We collected eight females from established study populations in eastern Delaware, U.S.A. (*M. g. nigrescens*) for female copulation solicitation assays to test female preferences for song. Each female was tested with eight unique stimulus sets (one set per female). We used natural song recorded from inland populations in Crawford County, Pennsylvania, U.S.A., and from coastal populations in Atlantic County, New Jersey, U.S.A. Female songbirds, including inland swamp sparrows (Anderson 2009), are sensitive to local songs and will discriminate between songs of local and nonlocal males (i.e. Baker 1982; Nowicki et al. 2002a, b; Nelson & Soha 2004; Danner et al. 2011). In the present study, we used songs from nonlocal populations to attempt to detect a subspecific preference, not dialect preferences, since females would have had no experience with any of the stimulus songs.

Copulation Solicitation Display

Adult female songbirds show positive responses to male song in nature by performing copulation solicitation displays, a courtship display given before and during copulation. We used the copulation solicitation display to test for song preferences in adult female swamp sparrows (following Searcy & Marler 1981) in three experiments (see below). Females were housed separately in cages and placed into individual anechoic chambers. During each experiment, only the focal female was exposed to playback of song over a loud speaker and her response was observed through a two-way mirror and videotaped. Two observers independently scored trials with 100% agreement between them. Each trial consisted of songs from one stimulus set played to an individual for 6 min at a rate of approximately five songs per minute. Each stimulus set consisted of 18 songs, with a single song type repeated nine times, followed by a second song type repeated nine times. Song stimuli were standardized to approximately 82 dB at distance of 1 m from the playback speaker. Females were tested once in the morning and once in the afternoon on the first day of testing. The order of presentation was randomized. Females heard no songs on the second day and were given the same stimulus on the third day, but in reverse order. Each stimulus was recorded from different males such that each female heard a unique set of stimuli. Copulation solicitation postures were scored on a scale of 0–3: 0 = no display;