vol. 197, no. 6 the american naturalist june 2021

Reproductive Character Displacement Drives Diversification of Male Courtship Songs in *Drosophila*

Roman Yukilevich[*]

Department of Biology, Union College, Schenectady, New York 12308

Submitted October 19, 2020; Accepted January 28, 2021; Electronically published April 23, 2021

Online enhancements: supplemental tables and figures. *Dryad data:* https://doi.org/10.5061/dryad.m63xsj41k.

abstract: Male secondary sexual traits are one of the most striking and diverse features of the animal kingdom. While these traits are often thought to evolve via sexual selection, many questions remain about their patterns of diversification and their role in speciation. To address these questions, I performed a comparative study of precopulatory male courtship songs of 119 *Drosophila* species across 10 distinct species groups. I related song divergence to genetic distances, geographic relationships, and sexual isolation between species. On the basis of pairwise Euclidean song distances, species groups typically retained their phylogenetic signal while species within groups diverged five times more in sympatry relative to allopatry, producing a pattern of reproductive character displacement. This occurred despite similar genetic distances in allopatry and sympatry, was exaggerated among younger species pairs, and was driven primarily by the parameter interpulse interval. While sexual isolation in sympatry was high even with low song divergence, these variables were correlated with each other and with increased divergence of female mating preferences in sympatry. The widespread pattern of character displacement implies that allopatric divergence due to processes like sexual selection are very slow relative to sympatric processes such as reinforcement and reproductive interference in driving song diversification across *Drosophila*.

Keywords: sexual selection, sexual isolation, species recognition, reinforcement, secondary sexual traits, noisy neighbors hypothesis.

Introduction

One of the most striking and diverse features of animals are their secondary sexual traits, such as sexual ornaments, plumage, courtship songs, and displays. These traits are mostly (but not exclusively; e.g., Edward and Chapman 2011) found in males, are unique to each species, and are involved in either male-male competition or attracting females to mate (Andersson 1994). Darwin (1859, 1871) was the first to describe these traits across animals and argue that they are products of sexual selection. Since then, two related questions have dominated the field: how do male secondary sexual traits diversify across lineages, and what is their role in speciation?

Most attention has focused on male traits involved in female choice (e.g., Lande 1981; West-Eberhard 1983; Andersson 1994; Coyne and Orr 2004; but see Lackey et al. 2018). Darwin's view that these traits diverge via sexual selection became widely accepted only in the 1980s with the development of sexual selection models, such as Fisherian runaway and good genes (Fisher 1930; Lande 1981; Kirkpatrick 1982; see also Paterson 1978; West-Eberhard 1983; Andersson 1994; Prum 2017). In principle, allopatric populations with limited or no gene flow can undergo sexual selection in different evolutionary directions and diverge in sexual communication systems. Additional processes can also drive sexual trait divergence in allopatry, including pleiotropic by-product of divergent ecological selection (e.g., Mayr 1963; Coyne and Orr 2004), sensory drive in different environments (Ryan 1990, 2018; Endler 1992; Cummings and Endler 2018), and mutation-order divergence (Mendelson et al. 2014).

Intriguingly, the role of sexual selection in evolution was not widely emphasized during the modern synthesis; instead, secondary sexual traits were often viewed as markers of species identity shaped by natural selection in sympatry (Dobzhansky 1937, 1970; Lack 1947, 1971; Alexander 1962; Mayr 1963; Bennet-Clark and Ewing 1970). This concept is often referred to as the species recognition hypothesis (but see Mendelson and Shaw 2012). Accordingly, male sexual traits are hypothesized to diverge via female choice when natural selection favors females to avoid (1) maladaptive hybridization during speciation (i.e., "reinforcement") or (2) wasting time and energy sexually interacting with sympatric species that no longer hybridize (i.e., the reproductive interference or noisy neighbors hypothesis; Butlin 1987; Otte 1989; Noor 1999; Gröning and Hochkirch 2008; Hoskin and Higgie 2010; Matute 2014, 2015).

Explaining divergence in secondary sexual traits remains one of the central goals in evolutionary biology because it relates to questions concerning the role of sexual selection, reinforcement, and ecological selection in speciation

---

[*] Email: yukilevr@union.edu.

Am. Nat. 2021. Vol. 197, pp. 690–707.

DOI: 10.1086/714046

(Panhuis et al. 2001; Servedio and Noor 2003; Mann and Seehausen 2011; Nosil 2012; Mendelson and Shaw 2012; Servedio and Boughman 2017; Kopp et al. 2018). The species recognition hypothesis predicts that sexual traits should diverge between partially or completely sympatric taxa. Therefore, if sexual traits are at least partially shaped by sympatric conditions, this may result in a pattern of reproductive character displacement (RCD; Brown and Wilson 1956; Walker 1974; Butlin 1989; Gröning and Hochkirch 2008; Pfennig and Pfennig 2009).

Since Brown and Wilson (1956), many have tried to test for RCD, typically at the level of populations, comparing allopatric versus sympatric populations of partially overlapping species pairs (for larger-scale studies, see below and Hollander et al. 2013). While such cases have been identified (Littlejohn 1965; Waage 1979; Sætre et al. 1997; Marshall and Cooley 2000; Höbel and Gerhardt 2003; Lemmon 2009), many studies failed to show RCD in zones of contact (Bigelow 1965; Walker 1974; Butlin 1987, 1989; Noor and Aquadro 1998; Marshall et al. 2002; Amézquita et al. 2006; Blyth et al. 2008; Yukilevich et al. 2016). Adding to this problem is the possibility that the pattern of RCD at the population level can be erased over time as a result of intraspecific gene flow between allopatric and sympatric populations (Noor 1997; Coyne and Orr 2004).

Other studies have searched for the pattern of RCD at the species level, comparing allopatric versus sympatric species pairs. This approach was used to study the evolution of plumage and male courtship songs in birds (Seddon 2005; Martin et al. 2010; Hudson and Price 2014; Tobias et al. 2014; Cooney et al. 2019). Many of the studies that explicitly correct for time of divergence have failed to find broad support for RCD (but see Martin et al. 2010). Instead, allopatric species pairs diverged at similar rates as their sympatric counterparts, and in Tobias et al. (2014) sympatric species even showed evidence of convergence. Hudson and Price (2014) suggested that the lack of RCD in male traits may reflect female birds narrowing their preference functions (i.e., "window of recognition") for preexisting male trait differences without changes in the male traits (sensu Butlin 1993; for a similar argument, see Noor 1999). Thus, both population- and species-level studies have so far provided inconsistent support for RCD.

The second major and complementary question is to understand the role of secondary sexual traits in speciation and sexual isolation (Panhuis et al. 2001; Coyne and Orr 2004; Ritchie 2007; Servedio and Boughman 2017). However, knowing that these traits are used in species identity would not necessarily indicate which process was responsible (Otte 1989; Ryan and Rand 1993; Mendelson and Shaw 2012). Moreover, secondary sexual traits may be composed of multiple components, which may diversify by different sexual and natural selection processes and therefore may vary in their relationship with sexual isolation (Saarikettu et al. 2005; Ryan 2018). Nevertheless, testing the relationship between such traits and sexual isolation is essential. It may show only a weak or no relationship with sexual isolation if, for instance, more ecologically relevant traits cause speciation (e.g., Servedio et al. 2011).

A limited number of case studies have directly implicated secondary sexual traits in sexual isolation, including pigment color in African cichlids (Wagner et al. 2012), courtship calls in crickets (Gray and Cade 2000; Mendelson and Shaw 2005) and frogs (Ryan and Rand 1993; Hoskin et al. 2005; Boul et al. 2007; Lemmon 2009; Ryan 2018), courtship songs in *Drosophila* (Bennet-Clark and Ewing 1969; Tomaru et al. 1995; Ritchie et al. 1999; Saarikettu et al. 2005; Yukilevich et al. 2016; but see Debelle et al. 2016), and ornamental plumage in birds (Sætre et al. 1997). Despite these case studies, the overall contribution of secondary sexual traits to speciation and sexual isolation across groups of taxa is not well understood.

To address the above questions at a large taxonomic comparative scale, one needs to study many species pairs of a taxonomic group for a well-characterized secondary sexual trait with information on phylogenetic relationships, genetic distances, geographical distributions, and reproductive isolation. The genus *Drosophila* provides an excellent opportunity for fulfilling these criteria, since much of the above-described data have been gathered since the seminal work of Coyne and Orr (1989, 1997; see Yukilevich 2012, 2013, 2014; Yukilevich and Paterson 2019). In the present study, I leveraged the above-described data set to perform a comparative study of *Drosophila* male courtship songs. I asked how songs diverge in allopatry and sympatry and tested their relationship to speciation.

During courtship, males of *Drosophila* typically use wing vibrations to produce bursts of sound to stimulate females to mate, referred to as "precopulatory courtship songs" (Ewing and Bennet Clark 1968; Tomaru and Yamada 2011). These songs are species specific, vary extensively across the genus, and are in some cases known to impact male mating success and species recognition (Bennet-Clark and Ewing 1969; Tomaru et al. 1995; Hoikkala et al. 1998; Ritchie et al. 1998, 1999; Yukilevich et al. 2016; Iglesias and Hasson 2017). Most notably, the parameter interpulse interval (IPI) in pulse songs (see below) is known to play a role in female mate choice between several species pairs (Bennet-Clark and Ewing 1969; Tomaru et al. 1995; Ritchie et al. 1999; Williams et al. 2001; Talyn and Dowse 2004; Saarikettu et al 2005; Debelle et al. 2014; Yukilevich et al. 2016). In other cases, mate choice depends on other song parameters, such as intrapulse frequency (IPF), cycles per pulse (CPP), period of pulse (POP), pulses per burst (PPB), and interburst interval (IBI; von Schilcher 1976; Noor and Aquadro 1998; Williams et al. 2001; Yamada et al. 2002; Saarikettu et al 2005). However, we know little about how these song parameters diverge and their overall contribution to sexual isolation. The only comparative work on *Drosophila* song evolution has been done in the *willistoni* and *buzzatii* groups (Ritchie and Gleason 1995; Gleason and Ritchie 1998; Oliveira et al. 2013). These studies found that songs diverged very rapidly, but the relationship to geography and sexual isolation was not explored.

To address these questions, I studied male courtship songs across 119 species spanning 10 distinct species groups, with 198 original and 72 phylogenetically corrected species pairs. Results showed that species groups typically retained their phylogenetic signal in song repertoire and in parameters such as IPI but did not always evolve predictably with time of divergence, especially in sympatry. However, songs were strongly affected by species geographical overlap when controlling for genetic distance, evolving roughly five times faster in sympatry over allopatry. This pattern of RCD was largely, but not exclusively, driven by divergence in IPI. Moreover, while sexual isolation in sympatry was high even with low song divergence, these variables were correlated with each other and with increased divergence in female mating preference in sympatry. I discuss these findings in the context of sexual selection, reinforcement, and reproductive interference and compare results to studies of birds.

Methods

Species and Song Type

I searched the literature on Web of Science and Google Scholar using the following keywords: "*Drosophila*" and "male courtship songs." Only studies that focused on precopulatory, wing-generated pulse and sine male courtship songs were included in the analysis (Tomaru and Yamada 2011). I excluded species with no male songs or with only postcopulatory male songs (e.g., Chen et al. 2019). Moreover, other male sounds were excluded because they were often generated by abdomen vibrations or could not be compared with other taxa, and in some cases they were excluded because it was unclear whether they were part of the courtship ritual (Hoy et al. 1988; Ritchie and Gleason 1995). Many Hawaiian species were excluded for these reasons. Finally, I did not study sounds made by females during courtship (e.g., rejection sounds or duetting; LaRue et al. 2015).

In total, the literature search resulted in 30 relevant publications, which totaled 119 species across 10 distinct species groups—*funebris*, *immigrans*, *quinaria*, *repleta*, *virilis*, *melanogaster*, *montium*, *obscura*, *willistoni*, and *planitibia*—spanning both subgenera *Drosophila* and *Sophophora*, each with 58 species, and 3 species from Hawaiian *Drosophila* (supplemental data set 1, available like the other data sets in the Dryad Digital Repository [https://doi.org/10.5061/dryad.m63xsj41k; Yukilevich 2021). This represented from 1% to 86% of all available species per group (table S1; tables S1–S6 are available online). From each study, I extracted the mean values for all available male courtship song parameters for each species (means based on 1–21 strains/species; see studies for details; supplemental data set 1).

The 119 species exhibited one of the three major song repertoires: repertoire 1 (a single primary pulse song), repertoire 2 (two types of pulse songs, "primary" and "secondary"), and repertoire 3 (a primary pulse song and a sine song; illustrated in fig. 1*A*; see below for descriptions). Species with two types of pulse songs and a sine song were not found. Additional differences in primary pulse song structure, such as K-H cycles, were not included (Kyriacou and Hall 1980; Clemens et al. 2018).

I defined the primary pulse song as the most frequent during courtship relative to the secondary pulse song. This applied to alternative definitions of pulse songs, sometimes referred to as high-repetition-rate (HRR) and low-repetition-rate (LRR) pulse songs, with HRR being primary and LRR being secondary songs (Chang and Miller 1978). Supplemental data set 1 also shows that the primary pulse song is virtually always the one with shorter IPI relative to the secondary pulse song, with only five exceptions out of 119 species (*pseudoananasae*, *yakuba*, *santomea*, *peninsularis*, and *equinoxialis*). Results of pairwise analyses were not affected by these exceptions because they were performed within species groups that share the same definitions. Moreover, I tested the robustness of the results by averaging the parameter values across primary and secondary pulse songs, which eliminates these designations (see average columns in supplemental data set 5). Finally, whenever more than one study described the species song, I averaged the parameter values while making sure the definitions of songs were consistent (see supplemental data set 1).

Specific Song Parameters

For individual song parameters, I followed standard definitions (table 1). Pulse songs are composed of cycles (fig. S1; figs. S1–S5 are available online). Thus, within a pulse, we define (1) the number of cycles per pulse (CPP), (2) the average period of cycle (POC), and (3) the period of pulse (POP), equal to CPP × POC, in milliseconds (fig. S1). The amount of time from one pulse to the next pulse is (4) the interpulse interval (IPI), measured in milliseconds, from the top of one pulse to top of the next pulse (fig. S1; Ewing and Bennet-Clark 1968). There are also two frequency parameters (table 1): one is (5) the intrapulse frequency (IPF), defined in hertz, which is the number of cycles per second within a pulse and is equal to the carrier (peak) frequency (fig. S1). IPF is measured by the inverse of the average period of the cycle of a pulse (i.e., the shorter the POC, the more cycles per second, and thus a higher

Table 1: Song parameters and their basic description

Abbreviation	Parameter	Measurement
CPP	Cycles per pulse	Count of cycles
POC	Period of cycle	Milliseconds
POP	Period of pulse	Milliseconds
IPI	Interpulse interval	Milliseconds
IPF	Intrapulse frequency	Hertz
Sine freq.	Sine song frequency	Hertz
POB	Period of burst	Milliseconds
PPB	Pulses per burst	Count of pulses
IBI	Interburst interval	Milliseconds

Note: See "Methods" for further details.

IPF; fig. S1). For species with sine songs, (6) the sine frequency (Hz) is defined the same way as IPF, as the inverse of the average POCs within the burst. Finally, (7) the period of burst (POB), measured in milliseconds, is the amount of time from the start of a burst to the end of a burst; (8) pulses per burst (PPB) is the number of pulses in a burst; and (9) the interburst interval (IBI), measured in milliseconds, is the time from the end of one song burst to the beginning of the next song burst (table 1). Because both primary and secondary pulse song parameters are included, there are 16 continuous pulse song parameters and one sine song parameter. Below I test for interdependencies between parameters.

Standardization of Song Parameters

To account for the qualitative difference in song repertoires (1, 2, and 3; see above), categorical presence and absence values of 1s and 0s were given to different songs depending on whether a species had a primary pulse song, a secondary pulse song, or a sine song. Since all species sang a primary pulse song, species could differ only in the presence of secondary pulse songs versus sine songs or in the absence of both (supplemental data set 1).

Once data for all relevant song parameters (2 categorical and 17 continuous) were gathered for all species, I performed a z-score normalization on each parameter to standardize the scale to a mean of 0 and a standard deviation of 1.0, using the following equation: (original value − mean of distribution)/standard deviation of distribution. I then performed log transformation to ensure that all parameters approximated a normal distribution.

Correlations between Song Parameters

I determined pairwise nonparametric Spearman's ρ correlations between all 19 standardized song parameters (supplemental data set 2). Given the 143 pairwise tests performed, after a Bonferroni correction the new significance threshold was $P = .00035$. Only 11 pairwise correlations were significant at this threshold, with eight of the significant correlations involving the same four parameters that were especially intercorrelated with others: primary and secondary pulse song cycles per pulse (CPP1 and CPP2) and period of cycle (POC1 and POC2; see supplemental data set 2). These four parameters were thus excluded from further analysis, resulting in two categorical and 13 continuous parameters. None of the other correlations were high and significant for other parameters (i.e., above $\rho = 0.75$). However, including all parameters or analyzing each parameter separately produced similar results (see below).

Temperature Corrections of Song Parameter Values

Virtually all studies recorded the ambient temperature during song recording, with an average temperature of 23.8°C (range: 19.0°–25.5°C; supplemental data set 1). These studies either temperature-corrected song parameter values (e.g., Ritchie and Gleason 1995) or maintained the same temperature throughout (e.g., Ewing 1979). I tested the relationship between temperature and all remaining continuous song parameters across all species. Four parameters produced significant regression coefficients with temperature (all were primary pulse song parameters: IPF, PPB, POP, and IBI; IPI had a negative trend that was not significant; table S2). This was expected because similar relationships have been observed within species (e.g., IPI had a negative relationship with temperature in all species studied in both subgenera; see table S3). Song variation across species may reflect not only biological differences but also variation in recording temperatures across studies.

Thus, I corrected for temperature across all species to eliminate the potential effect of temperature on the observed species measurements across disparate studies (supplemental data set 3). For each song parameter, each species has an observed y_o value. The basic equation for correction was

$$y_p = mx + b, \tag{1}$$

where y_p is the predicted song parameter value based on the relationship with recording temperature, m is the slope of the relationship between temperature and song parameter across species, x is the recording temperature, and b is the intersection of the relationship. The predicted y_p value for each species was then subtracted from the total mean ($\bar{y}$) of the song distribution across species (which was zero after z-score normalization), and the difference was added to the original parameter value y_o to get the temperature-corrected value (y_{tc}):

$$y_{tc} = y_o + (\bar{y} - y_p). \tag{2}$$

While analyses were based on temperature-corrected values (supplemental data set 3), the original data (supplemental data set 1) produced qualitatively similar results (not shown).

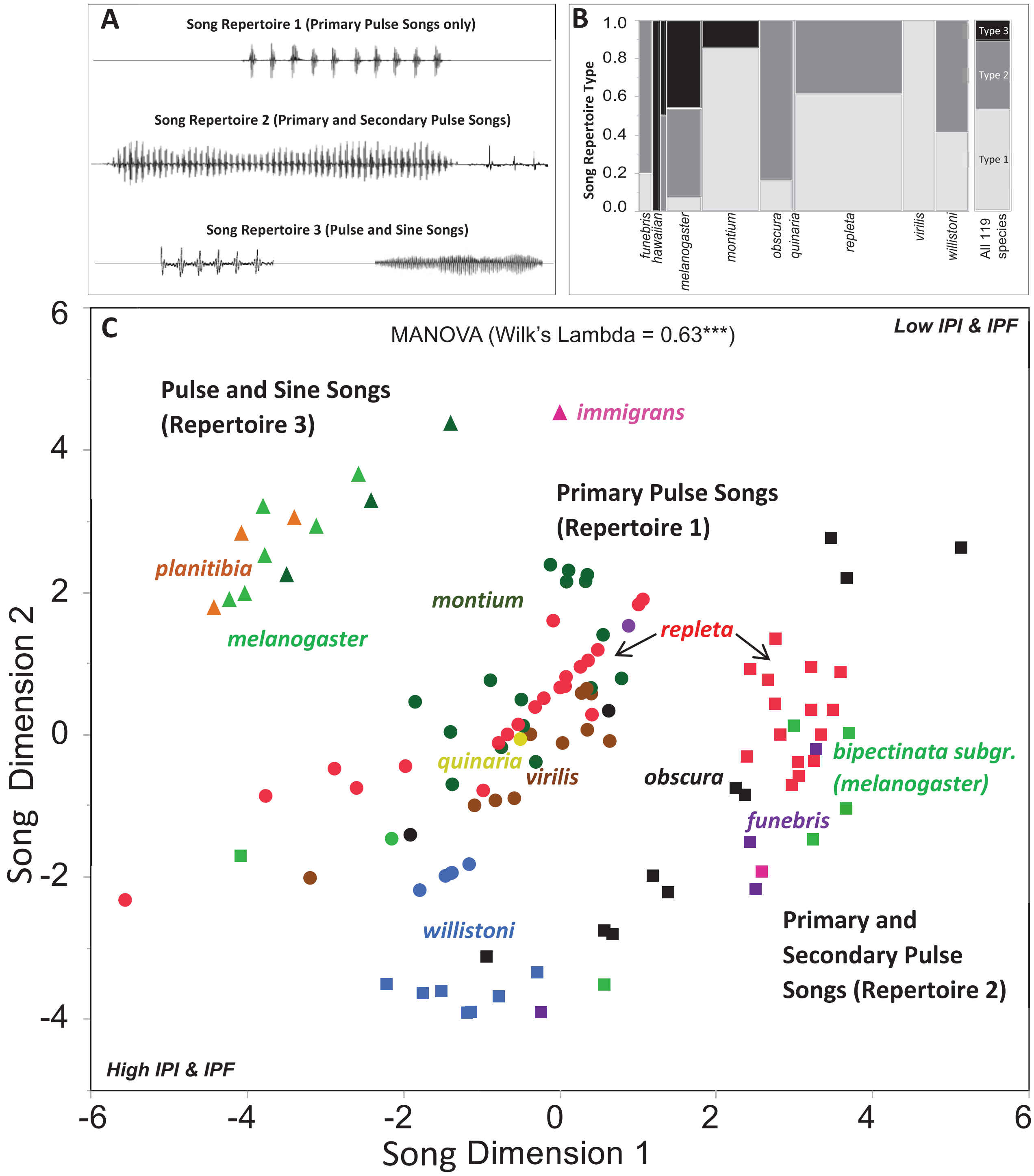

Figure 1: Representation and description of precopulatory male courtship songs of 119 species of the genus *Drosophila*. *A*, Examples of sound waves of three types of song repertoires found in these species, including primary pulse songs only (song repertoire 1), primary and secondary pulse songs (song repertoire 2), and pulse songs with sine songs (song repertoire 3). *B*, Distribution of three types of song repertoires across the genus overall and in each species group, with each bar representing the relative frequencies of each repertoire and width of bars representing the relative number of species included in each group. Song repertoire 1 = light gray; song repertoire 2 = dark gray; song repertoire 3 = black. *C*, Multidimensional scaling plot of 115 species courtships songs based on pairwise Euclidean distances across 15 independent song parameters (see "Methods" for details). Seven species or subspecies were excluded from this analysis because of missing interpulse interval (IPI) or intrapulse frequency

Euclidean Song Distances

Given that male courtship songs are composed of multiple song parameters, to determine species differences I used a composite Euclidean song distance measure (see below). This distance measure is best applicable for variables that are independent and based on the same scale (criteria that were satisfied above). It determines the minimum absolute song distance between species in a multiparameter "song space." Pairwise Euclidean song distances were calculated between all 119 available species based on 2 categorical and 13 continuous song parameters using the following Euclidean distance formula:

$$\text{composite Euclidean song distance}_{\text{sp.1}-\text{sp.1}} = \sqrt{\sum\nolimits_{i=1}^{n=j}\left(\left(x_{i,\text{sp.1}} - x_{i,\text{sp.2}}\right)^2 \times \left(\frac{j}{j_{\text{w/ data}}}\right)\right)}, \qquad (3)$$

where x_i is the value of each song parameter of each species, j is the total number of possible parameters between species, and $j_{\text{w/ data}}$ is the number of parameters with data present in both species.

Matlab software was used to calculate equation (3) for each species pair with following the function: Output = pdist(input file,@naneucdist), where "naneucdist" corrected for missing parameter values based on the ratio $j/j_{\text{w/ data}}$ (www.mathworks.com/help/stats/pdist.html; Eirola et al. 2013). The correction is important because of the additive nature of the Euclidean distance (i.e., adding differences across parameters). Otherwise, species pairs with more complete data would have greater distances. However, the $j/j_{\text{w/ data}}$ correction ratio was relatively minor and highly uniform across all species pairs (mean of ratio: 1.91; standard deviation of ratio: 0.71). Nevertheless, I found results to be highly similar when I instead averaged Euclidean song distances across all parameters (see below).

The total number of parameters that one uses to compare species pairs for missing values (j) must vary, since different song repertoire types have a different number of possible parameters. This is to avoid confounding missing song values with having categorical differences in song repertoire type. For comparisons where one species had song repertoire 1 or where one species had repertoire 2 and the other repertoire 3, $j = 8$ (2 categorical and 6 continuous); for comparisons where both species had song repertoire 2, $j = 14$ (2 categorical and 12 continuous); and for comparisons where both species had song repertoire 3, $j = 9$ (2 categorical and 7 continuous). I then generated a matrix for all species pairwise calculations using the following Matlab function: Matrix Output = squareform (Output) (supplemental data set 4).

I also averaged the standard Euclidean distances across all available song parameters per species pair (see supplemental data set 5). The Euclidean distance measure for each parameter was

$$\text{Euclidean song distance}_{\text{sp.1}-\text{sp.2}} = \sqrt{\left(x_{\text{sp.1}} - x_{\text{sp.2}}\right)^2}, \qquad (4)$$

where x is the value of a given song parameter of each species. Mahalanobis distances were not used here because the 15 song parameters included were not significantly correlated (see above), which in effect equates Euclidean and Mahalanobis measures (e.g., Varmuza and Filzmoser 2016). In addition, when data sets contain many missing parameter values across species, the inverse variance-covariance matrix needed to calculate Mahalanobis distances is impossible to solve unless one imputes over all the missing data, a far less preferred approach (Varmuza and Filzmoser 2016).

Multidimensional Scaling (MDS) of Euclidean Song Distances

After generating a 119-by-119 Euclidean song distance matrix (supplemental data set 4), I performed MDS using JMP software (ver. 13), an approach in multivariate data sets allowing one to visualize a distance matrix by representing samples in a low-dimensional space (Greenacre and Primicerio 2014). For this analysis I excluded seven taxa because of missing IPI or IPF parameter values: *D. trapezifrons*, *D. orosa*, *D. nigrospiracula*, *D. leontis*, and three *D. melanogaster* races. I then tested how each major MDS dimension correlates with the 15 song parameters across species. To test whether species groups differ from each other in MDS song space, I performed a MANOVA based on the first two MDS song dimensions using JMP software (ver. 13).

Genetic Distances and Time since Divergence

For the following data sets, I focused on comparisons within species groups (totaling 198 species pairs) to study how song

---

(IPF) song parameter values: *D. trapezifrons*, *D. orosa*, *D. nigrospiracula*, *D. leontis*, and three *D. melanogaster* races. Dimension 1 explains roughly 36% of total variance, and dimension 2 explains 20% of total variance in courtship songs across species (based on Matlab). Species with different song repertoires are designated as follows: circles = primary pulse songs (repertoire 1); squares = primary and secondary pulse songs (repertoire 2); triangles = pulse with sine songs (repertoire 3). Species belonging to different species groups are labeled by different colors: *funebris* = purple; *hawaiian planitibia* = orange; *immigrans* = pink; *melanogaster* = light green; *montium* = dark green; *quinaria* = yellow green; *obscura* = black; *repleta* = red; *virilis* = brown; *willistoni* = blue. $^{***}P < .001$.

divergence relates to recent speciation events. First, genetic distances within groups were previously compiled by Coyne and Orr (1989, 1997) and Yukilevich (2012) based on allozyme Nei's *D* measure. I also used data from Yukilevich and Peterson (2019), which determined pairwise estimated time of divergence (ETD) in millions of years (myr) with the TimeTree resource based on nucleotide sequence distances from published analyses (http://www.timetree.org/; see Kumar et al. 2005; Hedges et al. 2006, 2015; supplemental data set 5). TimeTree provided both median and overall ETD across all available studies for a given species pair. Because Nei's *D* and ETD were highly correlated (Yukilevich and Peterson 2019), most findings were based on ETD. For many analyses, I focused on relatively young species pairs (ETD of ≤3 myr). The goal was to study song divergence between species still undergoing speciation (i.e., partial reproductive isolation; see Yukilevich 2012). However, younger (≤1 myr) or older (≤5 myr) species pairs produced similar results (see below).

Geographical Relationships between Species

Estimates of geographical ranges of species were based on published range maps (Markow and O'Grady 2005; Yukilevich 2012, 2014; available at www.researchgate.net/publication/344209007_zip_file_for_all_range_mapszip). The range area of each species and the absolute range overlap between species pairs were determined in square kilometers using the Google Maps area calculator (http://www.daftlogic.com/projects-google-maps-area-calculator-tool.htm). The percentage of overlap for each species was equal to the absolute range overlap between species pairs divided by the absolute range size of that species. These values were averaged to obtain mean percentage of range overlap per species pair ("% sympatry"; supplemental data set S2). Range overlap does not capture possible microhabitat differences. While species ranges may vary in accuracy, this is unlikely to create a systematic bias.

Indexes of Sexual Isolation, Male and Female Mating Preferences, and Postzygotic Isolation

Sexual isolation within groups were taken from Coyne and Orr (1989, 1997) and Yukilevich (2012), based on multiple-choice and no-choice mating assays in the laboratory. Data on male and female mating preferences was taken from Yukilevich and Peterson (2019), based on single-pair no-choice mating assays in the laboratory. In brief, in the latter tests it was assessed whether a male courted a female in each vial and, if so, whether that female copulated during the observation period. For each species pair, the study determined the proportion of males courting conspecific versus heterospecific females and the proportion of females copulating with conspecific versus heterospecific males that courted females. Sexual isolation, male courtship preference, and female copulation preference indexes were based on the isolation index from Sobel and Chen (2014):

$$1 - 2 \times \left[\frac{\text{freq. heterospecifics}}{\text{freq. heterospecifics} + \text{freq. conspecifics}} \right]. \tag{5}$$

The postzygotic isolation index was taken from Coyne and Orr (1989) and Yukilevich (2012), based on the percentage of F_1 hybrid male and female sterility and/or inviability of both reciprocal crosses; the index ranged from 0, where both hybrid females and males are fertile and viable; to 0.5, where either hybrid females or males are sterile or inviable; to 1, where both hybrid sexes are either sterile or inviable (see supplemental data set 5 for all indexes).

Phylogenetic Correction for Species Pairs

Phylogenies within groups were based on either allozyme or nucleotide sequence data sets (Yukilevich 2012; Yukilevich and Peterson 2019; supplemental data set 6). I used the same weighted phylogenetic correction as in Fitzpatrick and Turelli (2006) by averaging original nonindependent values across phylogenetic nodes. The unweighted approach of Coyne and Orr (1989) produced qualitatively similar results (data not shown). Star phylogenies were averaged over all unresolved species pairs. After correction, three possible geographical categories resulted: pure allopatric, pure sympatric, and "mixed geography." The latter category arises because some phylogenetically independent contrasts include both sympatric and allopatric species pairs. After correction, 72 species pairs were phylogenetically independent, with 39 from the *Sophophora* subgenus and 33 from the *Drosophila* subgenus, including 32 sister species pairs (supplemental data set 6). Results were qualitatively similar using raw data (corrected analyses are shown below).

Pairwise Statistical Analyses

As in previous publications (Coyne and Orr 1989, 1997; Yukilevich 2012, 2013, 2014), genetic distance, sexual isolation, and male courtship songs were not normally distributed and could not be properly transformed (Sokal and Rohlf 1995). Thus, in keeping with prior work, I relied on nonparametric statistics to test correlations and associations within the species pair data set, including Spearman's rank correlation (ρ) and Kruskal-Wallis test, using JMP software (ver. 13). I adjusted the significance threshold for multiple testing whenever applicable using the Bonferroni correction.

Results

Diversification of Male Courtship Songs

I found that 53% of 119 species had song repertoire 1 (primary pulse only), 36% had repertoire 2 (two types of pulses), and only 11% had repertoire 3 (primary pulse with sine songs; fig. 1*B*). There was substantial variation in these percentages across groups (fig. 1*B*) and in pairwise song distances across the genus, visualized using an MDS plot (fig. 1*C*). The first major MDS dimension explained 36% of song variance and was positively correlated with the presence of secondary pulse song and negatively correlated with the IPI of both pulse songs and the presence of sine song (table S4).

The second dimension explained 20% of song variance, was positively correlated with the POP of primary song and the PPB of secondary song, and was negatively correlated with several parameters, including the IPF of both songs, the IPI of secondary pulse song, and the presence of secondary pulse song and sine songs (table S4). Thus, *Drosophila* species differ most in song repertoires and in such parameters as IPI, POP, PPB, and IPF. While some parameters between primary and secondary pulse songs were correlated (e.g., $\rho = 0.72$ between the IPI of primary and secondary songs), major song parameters such as IPI, IPF, and PPB were uncorrelated with each other (see supplemental data set 2).

Furthermore, the MDS plot revealed that songs significantly differed between species groups (MANOVA: Wilks's $\lambda = 0.63$, df $= 18$, $F = 2.89$, $P < .0001$). Within species groups, taxa mostly diverged in parameter values, with occasional differentiation in song repertoires (e.g., *melanogaster*; fig. 1*C*; see fig. S2 for songs within species groups).

Song Divergence in Relation to Genetic Distance within Groups

Overall Euclidean song distance was weakly but significantly correlated with both measures of genetic distances (Nei's *D* and ETD; fig. 2*A*, 2*B*). This result held for allopatric taxa only (blue markers). In contrast, sympatric pairs were significant only with Nei's *D*, not with ETD (fig. 2). The relationship with ETD varied among species groups: for groups with more than five pairs, song divergence was correlated with ETD within the *melanogaster*, *virilis*, *willistoni*, and *obscura* (young taxa only) groups but not within the *repleta* and *montium* groups (fig. S2). For specific song parameters, only primary song IPI and POP and song repertoire type were significantly associated with ETD in allopatry, and none were significant in sympatry (table S5; see fig. 2*C* for IPI). Thus, while song distance increased with genetic distance when considering all taxa, this was not typically the case in sympatry.

RCD in Songs Is Widespread

A key pattern of song evolution was its consistently greater divergence among sympatric relative to allopatric taxa (fig. 2; Kruskal-Wallis test: z-score $= 2.09$, $n = 72$, $P = .037$). Because the interest is on speciation and because song distances tend to increase with time of divergence (fig. 2), I focused on species pairs with an ETD of ≤3 myr (see "Methods"), which showed a highly significant pattern of RCD with virtually no overlap in song divergence between allopatry and sympatry (fig. 3*A*; Kruskal-Wallis test: $\chi^2 = 11.26$, $n = 27$, $P = .0036$; near-identical results were attained with averaging song distances across parameters; see "Methods"; data are in supplemental data set 6). The above-described threshold was chosen before the analysis, but lower and higher thresholds produced similar results (e.g., ETD of ≤1 myr: z-score $= 2.64$, $n = 13$, $P = .008$; ETD of ≤5 myr: z-score $= 2.88$, $n = 44$, $P = .004$).

Songs of taxa with an ETD of ≤3 myr represented six species groups and on average diverged about five times more in sympatry over allopatry (mean distances: sympatry $=$ 1.87, allopatry $= 0.35$; ratio $= 1.87/0.35 = 5.34$), amounting to 84% of all song divergence (mean sympatry divergence/total mean divergence $= 1.87/(1.87 + 0.35) = 84\%$; fig. 3*A*). The pattern of RCD remained significant among exclusively sister species pairs (fig. S3*A*; z-score $= 3.31$, $n = 17$, $P = .0027$) when analyzed in terms of percentage of geographical overlap (fig. S3*B*; $\rho = 0.51$, $n = 27$; $P = .007$) and was apparent in five of the six species groups with sufficient sample size (fig. S3*C*).

Importantly, the pattern of RCD was not a by-product of overall divergence time, since (1) sympatric pairs were not significantly older (using either Nei's *D* or ETD estimates) and did not have more postzygotic isolation than allopatric pairs (table 2), (2) there was no relationship between geographical overlap and ETD ($\rho = 0.25$, $n = 27$, $P > .05$), and (3) partial correlation between song distance and percentage of sympatric overlap remained significant when controlling for ETD (partial Pearson product-moment correlation $= 0.438$, $n = 27$, $P < .05$). Furthermore, the pattern of RCD occurred among the youngest and most incipient species pairs (ETD of ≤1 myr) despite their extremely weak postzygotic isolation (table S6).

To determine which parameter or parameters drove the above-described patterns, I tested for RCD for each song parameter separately. After correcting for multiple testing, primary song IPI was the only parameter to show significant RCD (table 3; also see figs. 2*C*, 3*B*), even though most other song parameters had similar trends of increasing divergence in sympatry (table 3). Qualitatively similar results were found for other ETD thresholds (data not shown). When I excluded primary song IPI distance from the composite Euclidean song distance, the pattern of RCD was

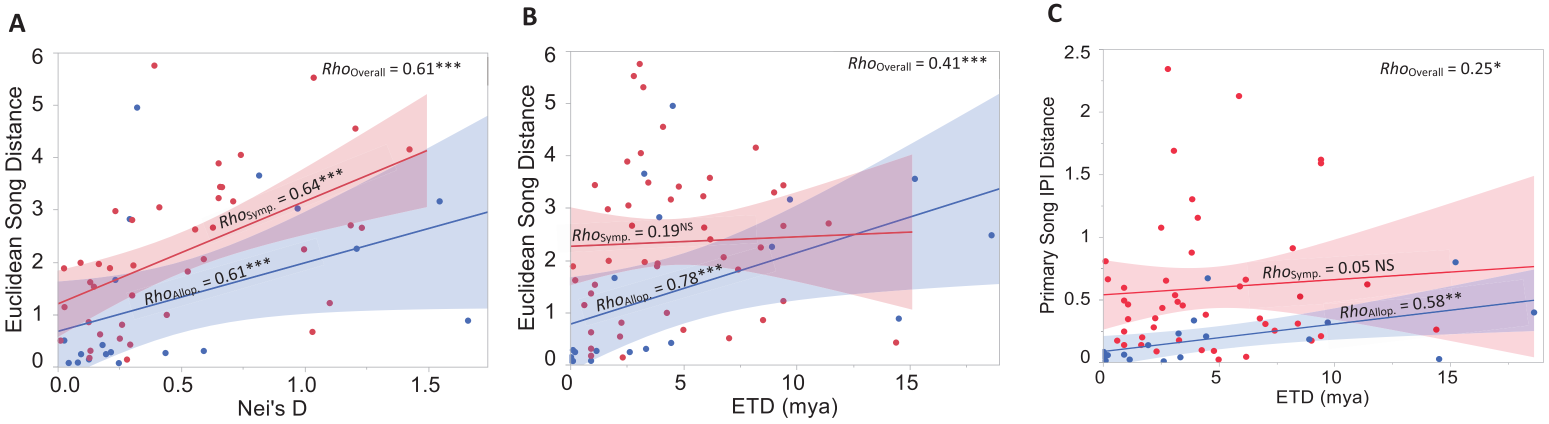

Figure 2: Relationship between Euclidean song distance and genetic distance for all corrected species pairs separated into allopatric taxa (blue circles) and partially (mixed) or completely sympatric corrected species pairs (red circles). *A*, Composite Euclidean song distance by Nei's *D*. *B*, Composite Euclidean song distance by estimated time of divergence (ETD). *C*, Primary song interpulse interval (IPI) Euclidean distance by ETD. mya = million years ago. NS = not significant; $^{*}P < .05$; $^{**}P < .01$; $^{***}P < .001$.

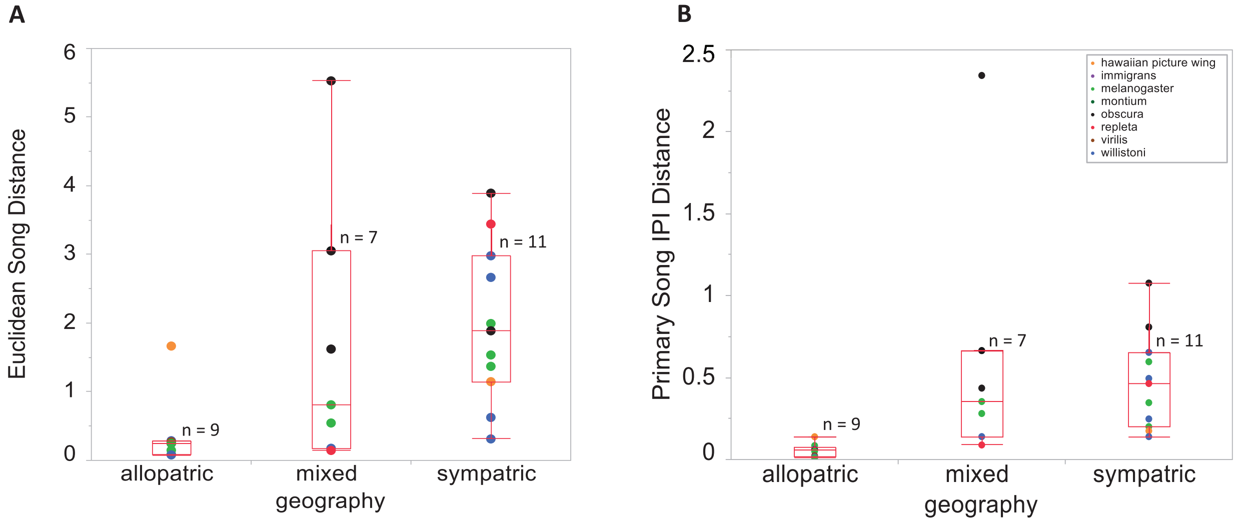

Figure 3: Euclidean song distance between allopatric, geographically mixed, and sympatric pairs with an estimated time of divergence of ≤3 million years. *A*, Composite Euclidean song distance. Kruskal-Wallis test: $\chi^2 = 11.26$, $P = .0036$. Post hoc Steel-Dwass test: significant only between allopatric and sympatric taxa at *z*-score = 3.34, $P = .0024$. *B*, Primary song interpulse interval (IPI) distance. Kruskal-Wallis test: $\chi^2 = 17.01$, $P = .0002$. Post hoc Steel-Dwass test: between allopatric and sympatric taxa at *z*-score = 3.72, $P = .0006$; between allopatric and mixed taxa at *z*-score = 2.17, $P = .0043$. Colors designate different species groups based on figure 1.

weaker but still significant (Kruskal-Wallis test: $\chi^2 = 7.56$, $n = 27$, $P = .02$). Thus, RCD in songs was largely but not completely driven by divergence in primary song IPI.

Complex Relationship between Song Divergence and Sexual Isolation

Taking all taxa into consideration, I found a positive significant relationship between song distance and sexual isolation (fig. 4*A*; $\rho_{\text{overall}} = 0.45$, $n = 63$, $P < .001$). To determine which song parameter or parameters were responsible, I tested the relationship between sexual isolation with each parameter. After correcting for multiple testing, only primary song IPI was significant (table 4; fig. 4*B*; $\rho = 0.58$, $n = 63$, $P < .001$).

When allopatric and sympatric taxa were analyzed separately, the above-described relationship applied only to allopatric taxa (fig. 4, blue markers). Because sexual isolation and songs were both highly correlated with ETD in allopatry (see above and fig. S4), their near-linear relationship

Table 2: Comparison of allopatric, geographically mixed, and sympatric corrected species pairs with an estimated time of divergence (ETD) of ≤3 million years (myr)

	All pairs			Only sister pairs		
	Sample size (*n*)	Mean (SD)	χ^2 (*P*)	Sample size (*n*)	Mean (SD)	χ^2 (*P*)
ETD:						
Allopatric	9	.78 (.95)	5.21 (.074)	8	.63 (.91)	2.22 (.33)
Mixed	7	1.88 (.95)		2	1.25 (1.49)	
Sympatric	11	1.26 (.79)		8	.99 (.52)	
Nei's *D*:						
Allopatric	9	.19 (.12)	2.40 (.30)	8	.18 (.06)	.32 (.85)
Mixed	7	.36 (.31)		2	.21 (.12)	
Sympatric	11	.28 (.25)		8	.21 (.06)	
Postzygotic isolation:						
Allopatric	9	.23 (.20)	1.23 (.54)	8	.19 (.08)	.86 (.65)
Mixed	7	.29 (.22)		2	.16 (.16)	
Sympatric	11	.34 (.30)		8	.29 (.08)	

Note: The left panel shows results for all pairs, and the right panel shows results for sister/sibling pairs only. Parameters include ETD (myr), Nei's *D*, and the postzygotic isolation index (0–1). Results for younger or older age groups also showed nonsignificant differences (data not shown).

Table 3: Test of reproductive character displacement for all song parameters

Song parameter Euclidian distance	Allopatry mean (n)	Mixed mean (n)	Sympatry mean (n)	Sympatric/ allopatric means	% divergence due to sympatry	χ^2	P
Song repertoire	.0 (9)	.30 (7)	.48 (11)	. . .	1.00	2.73	.26
IPI primary song	.06 (9)	.62 (7)	.47 (11)	7.83	.89	17.02	.0002
IPI secondary song	.12 (2)	.32 (2)	.50 (5)	4.17	.81	3.36	.19
IPF primary song	.35 (7)	.73 (6)	.52 (10)	1.49	.60	1.71	.43
IPF secondary song	.0 (2)	.36 (2)	.42 (5)	. . .	1.00	3.2	.20
Sine song frequency	.37 (1)	. . .	.74 (1)	2.00	.67	1	.32
POB primary song	.15 (2)	.37 (3)	1.22 (4)	8.13	.89	7	.03
POB secondary song	. . .	.64 (1)	.35 (2)	. . .	. . .	1.5	.22
PPB primary song	.10 (2)	.25 (3)	1.11 (5)	11.10	.92	6.91	.03
PPB secondary song	. . .	.06 (1)	.14 (3)	. . .	. . .	.2	.65
POP primary song	.04 (5)	.63 (6)	.34 (6)	8.50	.89	3.74	.15
POP secondary song	. . .	.33 (2)	.49 (2)	. . .	. . .	.6	.44

Note: Only corrected species pairs with an estimated time of divergence of ≤3 million years were included. n = number of species pairs. Allopatry was based only on allopatric species pairs, mixed mean was based on partially sympatric corrected pairs, and sympatric mean was based on completely sympatric pairs. The Wilcoxon/Kruskal-Wallis one-way test was performed. The parameter interburst interval was not tested because of missing data for relevant groups. Ratio and percent divergence calculations were based on sympatric mean relative to allopatric mean, with mixed mean not included. The Bonferroni-corrected P value given nine relevant tests between allopatry and sympatry was $P = .05/9 = .0056$. IPF = intrapulse frequency; IPI = interpulse interval; POB = period of burst; POP = period of pulse; PPB = pulses per burst.

likely reflects general divergence with time. In sympatry, sexual isolation indexes were very high even with low song divergence (red markers in fig. 4*A*) but were significantly correlated with primary song IPI divergence (red markers in fig. 4*B*; $\rho = 0.31, n = 45, P = .036$). Qualitatively similar results were observed for taxa with an ETD of ≤3 myr (data not shown).

Female Mating Preferences Are Strongly Correlated with Song Divergence in Sympatry

I further utilized data from Yukilevich and Peterson (2019) that separated sexual isolation into male courtship preferences and female mating preferences (see "Methods"). I found that divergence in male courtship preferences was

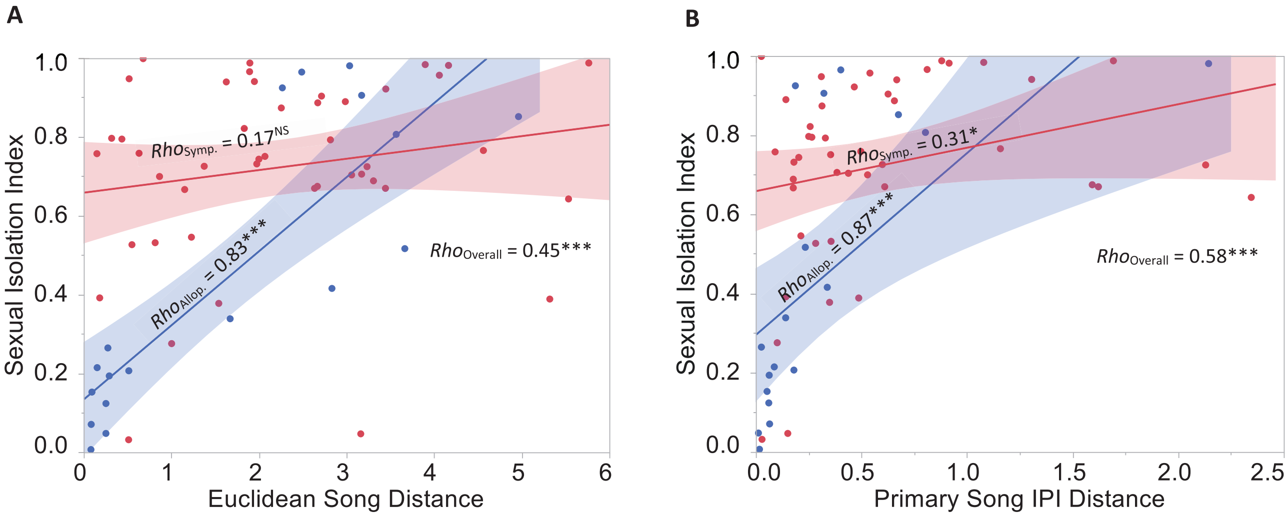

Figure 4: Relationship between sexual isolation index and composite Euclidean song distance (*A*) and primary song interpulse interval (IPI) distance (*B*), among allopatric pairs (blue markers) and partial or complete sympatric pairs (red markers). Qualitatively similar results were found when corrected species pairs were separated into allopatric, geographically mixed, and sympatric pairs (not shown). Trend lines and confidence intervals are shown for visual effect. Statistical relationships were based on nonparametric Spearman's ρ correlation. NS = not significant; $^{*}P < .05$; $^{***}P < .001$.

Table 4: Correlation between sexual isolation index and song parameter–specific Euclidean distances for all phylogenetically corrected species pairs

Song parameter Euclidean distance	Sample size (n)	Spearman's ρ coefficient	P
Song repertoire	63	.29	.02
IPI primary song	63	.58	<.0001
IPI secondary song	19	.43	.07
IPF primary song	48	.26	.07
IPF secondary song	18	.47	.05
Sine song frequency	6	.49	.33
POB primary song	21	.49	.03
POB secondary song	6	−.54	.27
PPB primary song	22	.39	.07
PPB secondary song	9	−.50	.17
POP primary song	32	.37	.04
POP secondary song	8	.10	.82
IBI primary song	9	.08	.83
IBI secondary song	5	.00	1.00

Note: Bonferroni-adjusted P value significance = .05/14 tests = .0036. IBI = interburst interval; IPF = intrapulse frequency; IPI = interpulse interval; POB = period of burst; POP = period of pulse; PPB = pulses per burst.

not correlated with either overall song distances or primary song IPI distances (fig. S5). In sharp contrast, divergence in female mating preferences was highly significantly correlated with both overall song distance (fig. 5*A*) and primary song IPI distance (fig. 5*B*). These results also held for sympatric taxa (red circles in fig. 5) and were observed when I controlled for ETD (partial correlation Pearson product-moment$_{\text{song IPI}\times\text{female pref.}} = 0.625$, $n = 19$, $P < .05$).

Finally, table 5 revealed that the magnitude of RCD was similar for songs, sexual isolation, and female mating preferences, accounting roughly for 82%–90% of all divergence among taxa with an ETD of ≤3 myr. Thus, these behavioral traits all responded to sympatric conditions in similar and parallel ways among *Drosophila*.

Discussion

Interest in how secondary sexual traits diversify and their role in speciation has been at the cornerstone of evolutionary biology (Lande 1981; West-Eberhard 1983; Andersson 1994; Coyne and Orr 2004; Prum 2017; Ryan 2018). This intersects questions about the role of sexual selection, reinforcement, and reproductive interference in shaping the sexual communication systems of animals (Butlin 1989; Panhous et al. 2001; Servedio and Noor 2003; Coyne and Orr 2004; Ritchie 2007; Nosil 2012; Servedio and Boughman 2017). Despite this, only a handful of studies explicitly related diversification of secondary sexual traits to genetic distances, geographical relationships, and sexual isolation across phylogenetic scales. The present work represents the first comprehensive study of precopulatory male courtship song diversification across *Drosophila* and provides novel observations about how these traits diversify and their relationship to speciation.

Results revealed that most species groups were significantly different in songs, with few groups having similar song repertoires. Within groups, species varied mostly in parameter values, especially IPI, IPF, and PPB. Interestingly, major song parameters were not correlated across species. This suggests that the fine-tuning of female acoustic receivers (ears) to male song IPF, as demonstrated in the *D. melanogaster* group (Riabinina et al. 2011), would not necessarily provide information about song IPI.

Moreover, songs tended to diverge predictably with time across allopatric taxa but were independent of divergence time in sympatry (especially using nuclear DNA as opposed to Nei's *D*, which was based on allozymes). This difference could possibly be explained by allozymes experiencing more selection and thus less gene flow in sympatry than sampled nuclear DNA. Regardless, this is the first indication that processes in sympatry causing divergence in songs may be different from those in allopatry. This was further highlighted by the much faster divergence of songs among sympatric relative to allopatric pairs, revealing a pattern of RCD. Songs diverged about five times faster in sympatry than in allopatry, and this pattern remained when controlling for time of divergence/genetic distance, was apparent in most groups, and was prominent among younger species pairs, including incipient cases. While sympatry does not necessarily imply syntopy, the above-described patterns suggest that most sympatric species are also likely syntopic.

Of the 15 song parameters, only IPI had significant RCD. This is consistent with several *Drosophila* case studies indicating that IPI is a key species recognition trait (Bennet-Clark and Ewing 1969; Tomaru et al. 1995; Ritchie et al. 1999; Saarikettu et al 2005; Yukilevich et al. 2016; R. Yukilevich, unpublished results). This was confirmed by the observation that IPI was the only song parameter significantly associated with sexual isolation, overall and specifically in sympatry. Divergence in other parameters, such as IPF, POB, and PPB, at most showed weak trends of RCD and weak effects on sexual isolation. However, because data on IPI was most extensive, perhaps larger sample sizes are needed to test for these effects in other parameters. Alternatively, other parameters may impact intraspecific mating success but may not be involved in species recognition (e.g., Saarikettu et al. 2005).

The above-described patterns are most consistent with reinforcement and reproductive interference driving increased divergence in male courtship songs in sympatry (along with female preferences; sensu Yukilevich and Peterson 2019). Reinforcement is expected to evolve after secondary contact against maladaptive hybridization (reviewed in Servedio and Noor 2003; Coyne and Orr 2004). Given that RCD in

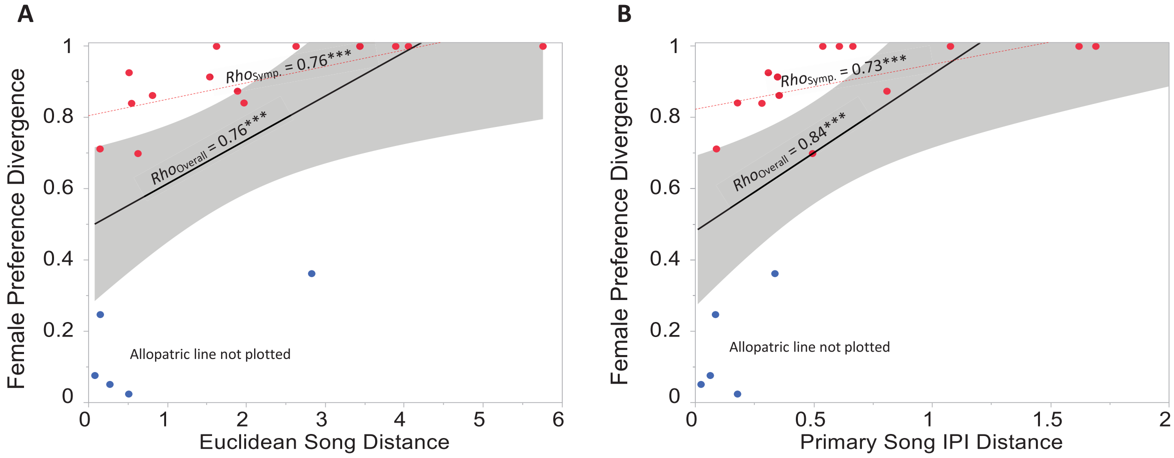

Figure 5: Relationship between female copulation preference divergence index and composite Euclidean song distance (*A*) and primary song interpulse interval (IPI) distance (*B*). Exclusively allopatric corrected species pairs are labeled as blue circles, and partially (mixed) or completely sympatric corrected species pairs are labeled as red circles. Note that the relationship was determined and plotted only for overall data and sympatric pairs, since allopatric pairs had an insufficient sample size. Qualitatively similar results were found when corrected species pairs were separated into allopatric, geographically mixed, and sympatric pairs (not shown). Trend lines and confidence intervals are shown for visual effect. Statistical relationships were based on nonparametric Spearman's ρ correlation. $^{***}P < .001$.

songs appears at early stages of divergence with weak postzygotic isolation, the type of selection against hybridization is unclear. Perhaps multiple processes are responsible, such as ecological or behavioral selection against hybrids (Coyne and Orr 1989, 1997; Yukilevich 2012; Yukilevich and Peterson 2019). Unfortunately, the ecology of most *Drosophila* is not known, and further work is necessary in this regard. Other interspecific interactions between sympatric species, such as reproductive interference, may also contribute to RCD, especially among more distantly related species (e.g., Otte 1989; Butlin 1989; Gröning and Hochkirch 2008). Natural selection to avoid interspecific matings may also trigger accelerated Fisherian runaway sexual selection in sympatry (Trivers 1972; Otte 1989; Andersson 1994). Conceivably, sexual selection can still partially explain rapid song divergence in sympatry.

However, pure divergent sexual selection in sympatry is an unlikely cause of RCD because sympatric taxa often exhibit some postzygotic isolation (e.g., Day 2000; Coyne and Orr 2004). Publication bias is also unlikely to have generated the pattern, since songs were studied without knowledge that the data would be used to make broad-scale comparisons. There is also no evidence that RCD in songs was driven by ecological character displacement in sympatry (e.g., Noor 1999). Alternatively, the Templeton effect argues that enhanced divergence in sympatry occurs because only highly divergent allopatric species can become sympatric without fusing back or becoming extinct (Templeton 1981; Noor

Table 5: Magnitude of reproductive character displacement when comparing partially and completely sympatric versus allopatric phylogenetically corrected pairs in overall courtship song, interpulse interval (IPI) of primary song, female mating preferences, and overall sexual isolation between young species pairs (estimated time of divergence of ≤3 million years)

	Overall courtship song	IPI (primary song)	Female mating preference	Overall sexual isolation
Sample size (*n*)	27	27	11	27
Sympatric mean	1.87	.53	.86	.74
Allopatric mean	.35	.06	.12	.16
Sympatric/allopatric means	5.34	8.83	7.17	4.63
% of divergence due to sympatry	.84	.90	.88	.82
χ^2 (Kruskal-Wallis test)	10.17	16.94	6.03	17.36
P (Kruskal-Wallis test)	.001	<.0001	.01	<.0001

Note: The sympatric mean groups together partially and completely sympatric phylogenetically corrected pairs. Results were even more different between sympatric and allopatric pairs if only completely sympatric pairs were used (data not shown).

1999; Coyne and Orr 2004; Martin et al. 2010). This predicts that sympatric taxa are a subset of allopatric taxa, but contrary to sympatry, there is not a single example of young allopatric species pairs (e.g., ETD of ≤1 myr) with high song divergence. While these hypotheses are less parsimonious than reinforcement or reproductive interference, it is possible that any one of them may have contributed to the overall observed pattern in some cases.

The results given above generally contrast with large-scale studies of birds that have provided inconsistent support for RCD in male songs and plumage (negative results: Hudson and Price 2014; Tobias et al. 2014; positive evidence: Sætre et al. 1997; Grant and Grant 2010; Martin et al. 2010; Cooney et al. 2019). In Tobias et al. (2014), it was even revealed that sexual traits showed convergence in sympatry. These studies suggest that allopatric conditions (e.g., via sexual selection) often play a major role in divergence of secondary sexual traits in birds. Given the present results, perhaps *Drosophila* songs are more essential in species recognition than in birds and/or may experience stronger stabilizing selection until impacted by direct selective pressure from congeners.

The widespread pattern of song RCD in *Drosophila* is surprising, since such patterns at the population level are not found where songs contribute to sexual isolation (Noor and Aquadro 1998; Blyth et al. 2008; Yukilevich et al. 2016; Poikela et al. 2019). Instead, in the cases described above, song parameters such as IPI are highly divergent in both sympatric and allopatric populations. This may occur if RCD in songs at the population level is quickly erased over time, resulting in species-wide character displacement (Walker 1974). Theoretical work shows that (1) pure divergent sexual selection erase male sexual trait differences in the face of gene flow without additional factors (Servedio and Bürger 2014) and (2) mating preferences and male sexual traits selected in sympatry by reinforcement spread easily into allopatry without ecological selection (Yukilevich and Aoki 2016). The speed of this spread depends on rates of intraspecific gene flow, which are high in many *Drosophila* (e.g., Jones et al. 1981; Wang and Hey 1996; Yukilevich et al. 2018). Thus, RCD in songs may often be quickly erased at the population level but can still be uncovered at the species level.

However, other prezygotic isolation traits in *Drosophila*, such as cuticular hydrocarbons and postmating prezygotic barriers, have been found to show RCD at the population level in several young species pairs (Higgie et al. 2000; Matute 2010; Dyer et al. 2014; Poikela et al. 2019). Perhaps, in contrast to songs, other sexual traits may experience divergent ecological selection preventing homogenization between allopatric and sympatric populations (e.g., Higgie et al. 2000).

Another surprising result is the very slow divergence of songs in allopatry, since such divergence is not constrained by homogenizing gene flow. In principle, allopatric divergence can occur by sexual selection, ecological pleiotropy, sensory drive, and mutation-order divergence (Mayr 1963; Lande 1981; Uyeda et al. 2009; Mendelson et al. 2014). Under experimental laboratory conditions, *Drosophila* songs respond to increasing sexual selection (Snook et al. 2005). However, considering the present results, it appears that the above-described processes in nature are either very slow or hampered by other constraints. For instance, if song divergence in allopatry reflects divergence in neutral female preferences via genetic drift, this would be a very slow process in large *Drosophila* populations. Further work on allopatric island endemics, including Hawaiian *Drosophila*, would be particularly useful.

Finally, it is interesting that sexual isolation in sympatry was very high even in cases where song divergence was low. This did not occur in allopatry, where these variables had a roughly linear relationship with each other and with time of divergence. The most obvious explanation is that speciation in sympatry involves more than just divergence of songs, such as divergence in male courtship preferences (e.g., Coyne et al. 1994; Shahandeh et al. 2018; Yukilevich and Peterson 2018) and other sexual traits, such as cuticular hydrocarbons (Cobb and Jallon 1990; Higgie et al. 2000; Chung et al. 2014; Dyer et al. 2014). This is consistent with song divergence in sympatry showing a more significant relationship with divergence in female mating preferences than with overall sexual isolation.

However, there may be a more subtle explanation as well. Noor (1999) proposed that enhanced sexual isolation in sympatry may be driven by the narrowing of female preference functions for preexisting male trait differences. Hudson and Price (2014) have argued that this can explain the difference between nonhybridizing and hybridizing sympatric bird species without RCD in male sexual traits. If this occurs in *Drosophila*, it may further explain why sexual isolation increases in sympatry even in species pairs with low song divergence. In total, the present study highlights these large-scale relationships and furthers our understanding of how secondary sexual traits evolve and diversify in nature.

Acknowledgments

I thank R. Butlin, W. Etges, L. Fleishman, J. Gleason, M. Ritchie, and K. Shaw for helpful comments on the manuscript. I thank R. Hurl for statistical advice. The author has no conflict of interest. This work was partially funded by National Science Foundation grant 1655935.

Data and Code Availability

Data sets are available from the Dryad Digital Repository (https://doi.org/10.5061/dryad.m63xsj41k; Yukilevich 2021).

Literature Cited

Alexander, R. D. 1962. Evolutionary change in cricket acoustical communication. Evolution 16:443–467.

Amézquita, A., W. Hödl, A. P. Lima, L. Castellanos, L. Erdtmann, and M. C. De Araujo. 2006. Masking interference and the evolution of the acoustic communication system in the Amazonian dendrobatid frog *Allobates femoralis*. Evolution 60:1874–1887.

Andersson, M. 1994. Sexual selection. Princeton University Press, Princeton, NJ.

Bennet-Clark, H. C., and A. W. Ewing. 1969. Pulse interval as a critical parameter in the courtship song of *Drosophila melanogaster*. Animal Behavior 17:755–759.

———. 1970. The love song of the fruit fly. Scientific American 223:84–93.

Bigelow, R. S. 1965. Hybrid zones and reproductive isolation. Evolution 19:449–458.

Blyth, J. E., D. Lachaise, and M. G. Ritchie. 2008. Divergence in multiple courtship song traits between *Drosophila santomea* and *D. yakuba*. Ethology 114:728–736.

Boul, K. E., W. C. Funk, C. R. Darst, D. C. Cannatella, and M. J. Ryan. 2007. Sexual selection drives speciation in an Amazonian frog. Proceedings of the Royal Society B 274:399–406.

Brown, W. L., and E. O. Wilson. 1956. Character displacement. Systematic Zoology 5:49–64.

Butlin, R. K. 1987. Speciation by reinforcement. Trends in Ecology and Evolution 2:8–13.

———. 1989. Reinforcement of premating isolation. Pages 158–179 *in* D. Otte and J. A. Endler, eds. Speciation and its consequences. Sinauer, Sunderland, MA.

———. 1993. The variability of mating signals and preferences in the brown planthopper, *Nilaparvata lugens* (*Homoptera*: *Delphacidae*). Journal of Insect Behavior 6:125–140.

Chang, H. C., and D. D. Miller. 1978. Courtship and mating sounds in species of the *Drosophila affinis* subgroup. Evolution 32:540–550.

Chen, A. L., C. C. Chen, T. Katoh, T. K. Katoh, M. Watada, M. J. Toda, M. G. Ritchie, and S. Y. Wen. 2019. Evolution and diversity of the courtship repertoire in the *Drosophila montium* species group (*Diptera*: *Drosophilidae*). Journal of Evolutionary Biology 32:1124–1140.

Chung, H., D. W. Loehlin, H. D. Dufour, K. Vacarro, J. G. Millar, and S. B. Carroll. 2014. A single gene affects both ecological divergence and mate choice in *Drosophila*. Science 343:1148–1151.

Clemens, J., P. Coen, F. A. Roemschied, T. D. Pereira, D. Mazumder, D. E. Aldarondo, D. A. Pacheco, and M. Murthy. 2018. Discovery of a new song mode in *Drosophila* reveals hidden structure in the sensory and neural drivers of behavior. Current Biology 28:2400–2412.

Cobb, M., and J. M. Jallon. 1990. Pheromones, mate recognition and courtship stimulation in the *Drosophila melanogaster* species subgroup. Animal Behaviour 39:1058–1067.

Cooney, C. R., Z. K. Varley, L. O. Nouri, C. J. Moody, M. D. Jardine, and G. H. Thomas. 2019. Sexual selection predicts the rate and direction of colour divergence in a large avian radiation. Nature Communications 10:1–9.

Coyne, J. A., A. P. Crittenden, and K. Mah. 1994. Genetics of a pheromonal difference contributing to reproductive isolation in *Drosophila*. Science 265:1461–1464.

Coyne, J. A., and H. A. Orr. 1989. Patterns of speciation in *Drosophila*. Evolution 43:362–381.

———. 1997. Patterns of speciation in *Drosophila* revisited. Evolution 51:295–303.

———. 2004. Speciation. Sinauer, Sunderland, MA.

Cummings, M. E., and J. A. Endler. 2018. 25 years of sensory drive: the evidence and its watery bias. Current Zoology 64:471–484.

Darwin, C. 1859. On the origin of species by means of natural selection, or the preservation of favoured races in the struggle for life. J. Murray, London.

———. 1871. The descent of man, and selection in relation to sex. J. Murray, London.

Day, T. 2000. Sexual selection and the evolution of costly female preferences: spatial effects. Evolution 54:715–730.

Debelle, A., M. G. Ritchie, and R. R. Snook. 2014. Evolution of divergent female mating preference in response to experimental sexual selection. Evolution 68:2524–2533.

———. 2016. Sexual selection and assortative mating: an experimental test. Journal of Evolutionary Biology 29:1307–1316.

Dobzhansky, T. 1937. Genetics and the origins of species. Columbia University Press, New York.

———. 1970. The genetics of the evolutionary process. Columbia University Press, New York.

Dyer, K. A., B. E. White, J. L. Sztepanacz, E. R. Bewick, and H. D. Rundle. 2014. Reproductive character displacement of epicuticular compounds and their contribution to mate choice in *Drosophila subquinaria* and *Drosophila recens*. Evolution 68:1163–1175.

Edward, D. A., and T. Chapman. 2011. The evolution and significance of male courtship preferences. Trends in Ecology and Evolution 26:647–654.

Eirola, E., G. Doquire, M. Verleysen, and A. Lendasse. 2013. Distance estimation in numerical data sets with missing values. Information Sciences 240:115–128.

Endler, J. A. 1992. Signals, signal conditions, and the direction of evolution. American Naturalist 139:S125–S153.

Ewing, A. W. 1979. Complex courtship songs in the *Drosophila funebris* species group: escape from an evolutionary bottleneck. Animal Behaviour 27:343–349.

Ewing, A. W., and H. C. Bennet-Clark. 1968. The courtship songs of *Drosophila*. Behaviour 31:288–301.

Fisher, R. A. 1930. The genetical theory of natural selection. Oxford University Press, Oxford.

Fitzpatrick, B. M., and M. Turelli. 2006. The geography of mammalian speciation: mixed signals from phylogenies and range maps. Evolution 60:601–615.

Gleason, J. M., and M. G. Ritchie. 1998. Evolution of courtship song and reproductive isolation in the *Drosophila willistoni* species complex: do sexual signals diverge the most quickly? Evolution 52:1493–1500.

Grant, B. R., and P. R. Grant. 2010. Songs of Darwin's finches diverge when a new species enters the community. Proceedings of the National Academy of Sciences of the USA 107:20156–20163.

Gray, D. A, and W. H. Cade. 2000. Sexual selection and speciation in field crickets. Proceedings of the National Academy of Sciences of the USA 97:14449–14454.

Greenacre, M., and R. Primicerio. 2014. Multivariate analysis of ecological data. Fundación BBVA, Bilbao.

Gröning, J., and A. Hochkirch. 2008. Reproductive interference between animal species. Quarterly Review of Biology 83:257–282.

Hedges, S. B., J. Dudley, and S. Kumar. 2006. TimeTree: a public knowledge-base of divergence times among organisms. Bioinformatics 22:2971–2972.

Hedges, S. B., J. Marin, M. Suleski, M. Paymer, and S. Kumar. 2015. Tree of life reveals clock-like speciation and diversification. Molecular Biology and Evolution 32:835–845.

Higgie, M., S. Chenoweth, and M. W. Blows. 2000. Natural selection and the reinforcement of mate recognition. Science 290:519–521.

Höbel, G., and H. C. Gerhardt. 2003. Reproductive character displacement in the acoustic communication system of green tree frogs (*Hyla cinerea*). Evolution 57:894–904.

Hoikkala, A., J. Aspi, and L. Suvanto. 1998. Male courtship song frequency as an indicator of male genetic quality in an insect species, *Drosophila montana*. Proceedings of the Royal Society B 265:503–508.

Hollander, J., C. M. Smadja, R. K. Butlin, and D. G. Reid. 2013. Genital divergence in sympatric sister snails. Journal of Evolutionary Biology 26:210–215.

Hoskin, C. J., and M. Higgie. 2010. Speciation via species interactions: the divergence of mating traits within species. Ecology Letters 13:409–420.

Hoskin, C. J., M. Higgie, K. R. McDonald, and C. Moritz. 2005. Reinforcement drives rapid allopatric speciation. Nature 437:1353–1356.

Hoy, R. R., A. Hoikkala, and K. Kaneshiro. 1988. Hawaiian courtship songs: evolutionary innovation in communication signals of *Drosophila*. Science 240:217–219.

Hudson, E. J., and T. D. Price. 2014. Pervasive reinforcement and the role of sexual selection in biological speciation. Journal of Heredity 105:821–833.

Iglesias, P. P., and E. Hasson. 2017. The role of courtship song in female copulation preferences in South American cactophilic *Drosophila*. PLoS ONE 12:e0176119.

Jones, J. S., S. H. Bryant, R. C. Lewontin, J. A. Moore, and T. Prout. 1981. Gene flow and the geographical distribution of a molecular polymorphism in *Drosophila pseudoobscura*. Genetics 98:157–178.

Kirkpatrick, M. 1982. Sexual selection and the evolution of female choice. Evolution 36:1–12.

Kopp, M., M. R. Servedio, T. C. Mendelson, R. J. Safran, R. L. Rodríguez, M. E. Hauber, E. C. Scordato, et al. 2018. Mechanisms of assortative mating in speciation with gene flow: connecting theory and empirical research. American Naturalist 191:1–20.

Kumar, S., A. Filipski, V. Swarna, A. Walker, and S. B. Hedges. 2005. Placing confidence limits on the molecular age of the human-chimpanzee divergence. Proceedings of the National Academy of Sciences of the USA 102:18842–18847.

Kyriacou, C. P., and J. C. Hall. 1980. Circadian rhythm mutations in *Drosophila melanogaster* affect short-term fluctuations in the male's courtship song. Proceedings of the National Academy of Sciences of the USA 77:6729–6733.

Lack, D. 1947. Darwin's finches. Harper, New York.

———. 1971. Ecological isolation in birds. Harvard University Press, Cambridge, MA.

Lackey, A. C. R., M. D. Martin, and R. M. Tinghitella. 2018. Male competition and speciation: expanding our framework for speciation by sexual selection. Current Zoology 64:69–73.

Lande, R. 1981. Models of speciation by sexual selection on polygenic traits. Proceedings of the National Academy of Sciences of the USA 78:3721–3725.

LaRue, K. M., J. Clemens, G. J. Berman, and M. Murthy. 2015. Acoustic duetting in *Drosophila virilis* relies on the integration of auditory and tactile signals. eLife 4:e07277.

Lemmon, E. M. 2009. Diversification of conspecific signals in sympatry: geographic overlap drives multidimensional reproductive character displacement in frogs. Evolution 63:1155–1170.

Littlejohn, M. J. 1965. Premating isolation in the *Hyla ewingi* complex (Anura: Hylidae). Evolution 19:234–243.

Maan, M. E., and O. Seehausen. 2011. Ecology, sexual selection and speciation. Ecology Letters 14:591–602.

Markow, T. A., and P. O'Grady. 2005. *Drosophila*: a guide to species identification and use. Elsevier, Amsterdam.

Marshall, D. C., and J. R. Cooley. 2000. Reproductive character displacement and speciation in periodical cicadas, with description of a new species, 13-year *Magicicada neotredecim*. Evolution 54:1313–1325.

Marshall, J. L., M. L. Arnold, and D. J. Howard. 2002. Reinforcement: the road not taken. Trends in Ecology and Evolution 17:558–563.

Martin, P. R., R. Montgomerie, and S. C. Lougheed. 2010. Rapid sympatry explains greater color pattern divergence in high latitude birds. Evolution 64:336–347.

Matute, D. R. 2010. Reinforcement of gametic isolation in *Drosophila*. PLoS Biology 8:e1000341.

———. 2014. The magnitude of behavioral isolation is affected by characteristics of the mating community. Ecology and Evolution 4:2945–2956.

———. 2015. Noisy neighbors can hamper the evolution of reproductive isolation by reinforcing selection. American Naturalist 185:253–269.

Mayr, E. 1963. Animal species and evolution. Belknap, Cambridge, MA.

Mendelson, T. C., M. D. Martin, and S. M. Flaxman. 2014. Mutation-order divergence by sexual selection: diversification of sexual signals in similar environments as a first step in speciation. Ecology letters 17:1053–1066.

Mendelson, T. C., and K. L. Shaw. 2005. Rapid speciation in an arthropod. Nature 433:375–376.

———. 2012. The (mis)concept of species recognition. Trends in Ecology and Evolution 27:421–427.

Noor, M. A. 1997. How often does sympatry affect sexual isolation in *Drosophila*? American Naturalist 149:1156–1163.

———. 1999. Reinforcement and other consequences of sympatry. Heredity 83:503–508.

Noor, M. A., and C. F. Aquadro. 1998. Courtship songs of *Drosophila pseudoobscura* and *D. persimilis*: analysis of variation. Animal Behaviour 56:115–125.

Nosil, P. 2012. Ecological speciation. Oxford University Press, Oxford.

Oliveira, C. C., M. H. Manfrin, F. D. M. Sene, and W. J. Etges. 2013. Evolution of male courtship songs in the *Drosophila buzzatii* species cluster. Pages 137–164 *in* P. Michalak, ed. Speciation: natural processes, genetics and biodiversity. Nova Science, New York.

Otte, D. 1989. Speciation in Hawaiian crickets. Pages 482–526 *in* D. Otte and J. A. Endler, eds. Speciation and its consequences. Sinauer, Sunderland, MA.

Panhuis, T. M., R. Butlin, M. Zuk, and T. Tregenza. 2001. Sexual selection and speciation. Trends in Ecology and Evolution 16:364–371.

Paterson, H. E. H. 1978. More evidence against speciation by reinforcement. South African Journal of Science 74:369–371.

Pfennig, K., and D. Pfennig. 2009. Character displacement: ecological and reproductive responses to a common evolutionary problem. Quarterly Review of Biology 84:253–276.

Poikela, N., J. Kinnunen, M. Wurdack, H. Kauranen, T. Schmitt, M. Kankare, R. R. Snook, and A. Hoikkala. 2019. Strength of sexual and postmating prezygotic barriers varies between sympatric populations with different histories and species abundances. Evolution 73:1182–1199.

Prum, R. O. 2017. The evolution of beauty: how Darwin's forgotten theory of mate choice shapes the animal world—and us. Anchor, New York.

Riabinina, O., M. Dai, T. Duke, and J. T. Albert. 2011. Active process mediates species-specific tuning of *Drosophila* ears. Current Biology 21:658–664.

Ritchie, M. G. 2007. Sexual selection and speciation. Annual Review of Ecology, Evolution, and Systematics 38:79–102.

Ritchie, M. G., E. J. Halsey, and J. M. Gleason. 1999. *Drosophila* song as a species-specific mating signal and the behavioural importance of Kyriacou & Hall cycles in *D. melanogaster* song. Animal Behaviour 58:649–657.

Ritchie, M. G., and J. M. Gleason. 1995. Rapid evolution of courtship song pattern in *Drosophila willistoni* sibling species. Journal of Evolutionary Biology 8:463–479.

Ritchie, M. G., R. M. Townhill, and A. Hoikkala. 1998. Female preference for fly song: playback experiments confirm the targets of sexual selection. Animal Behaviour 56:713–717.

Ryan, M. J. 1990. Sexual selection, sensory systems and sensory exploitation. Oxford Surveys in Evolutionary Biology 7:157–195.

———. 2018. A taste for the beautiful: the evolution of attraction. Princeton University Press, Princeton, NJ.

Ryan, M. J., and A. S. Rand. 1993. Species recognition and sexual selection as a unitary problem in animal communication. Evolution 47:647–657.

Saarikettu, M., J. O. Liimatainen, and A. Hoikkala. 2005. The role of male courtship song in species recognition in *Drosophila montana*. Behavior Genetics 35:257–263.

Sætre, G.-P., T. Moum, S. Bures, M. Kra, M. Adamjank, and J. Moreno. 1997. A sexually selected character displacement in flycatchers reinforces premating isolation. Nature 387:589–592.

Seddon, N. 2005. Ecological adaptation and species recognition drives vocal evolution in neotropical suboscine birds. Evolution 59:200–215.

Servedio, M. R., and J. W. Boughman. 2017. The role of sexual selection in local adaptation and speciation. Annual Review of Ecology, Evolution, and Systematics 48:85–109.

Servedio, M. R., and R. Bürger. 2014. The counterintuitive role of sexual selection in species maintenance and speciation. Proceedings of the National Academy of Sciences of the USA 11:8113–8118.

Servedio, M. R., and M. A. Noor. 2003. The role of reinforcement in speciation: theory and data. Annual Review of Ecology, Evolution, and Systematics 34:339–364.

Servedio, M. R., G. S. Van Doorn, M. Kopp, A. M. Frame, and P. Nosil. 2011. Magic traits in speciation: "magic" but not rare? Trends in Ecology and Evolution 26:389–397.

Shahandeh, M. P., A. Pischedda, and T. L. Turner. 2018. Male mate choice via cuticular hydrocarbon pheromones drives reproductive isolation between *Drosophila* species. Evolution 72:123–135.

Snook, R. R., A. Robertson, H. S. Crudgington, and M. G. Ritchie. 2005. Experimental manipulation of sexual selection and the evolution of courtship song in *Drosophila pseudoobscura*. Behavior Genetics 35:245–255.

Sobel, J. M., and G. F. Chen. 2014. Unification of methods for estimating the strength of reproductive isolation. Evolution 68:1511–1522.

Sokal, R. R., and F. J. Rohlf. 1995. Biometry: the principles and practice of statistics in biological research. 3rd ed. Freeman, New York.

Talyn, B. C., and H. B. Dowse. 2004. The role of courtship song in sexual selection and species recognition by female *Drosophila melanogaster*. Animal Behaviour 68:1165–1180.

Templeton, A. R. 1981. Mechanisms of speciation—a population genetic approach. Annual Review of Ecology and Systematics 12:23–48.

Tobias, J., C. Cornwallis, E. Derryberry, S. Claramunt, R. T. Brumfield, and N. Seddon. 2014. Species coexistence and the dynamics of phenotypic evolution in adaptive radiation. Nature 506:359–363.

Tomaru, M., H. Matsubayashi, and Y. Oguma. 1995. Heterospecific inter-pulse intervals of courtship song elicit female rejection in *Drosophila biauraria*. Animal Behaviour 50:905–914.

Tomaru, M., and H. Yamada. 2011. Courtship of *Drosophila*, with a special interest in courtship songs. Low Temperature Science 69:61–85.

Trivers, R. 1972. Parental investment and sexual selection. Pages 136–179 *in* B. Campbell, ed. Sexual selection and the descent of man. Aldine, Chicago.

Uyeda, J. C., S. J. Arnold, P. A. Hohenlohe, and L. S. Mead. 2009. Drift promotes speciation by sexual selection. Evolution 63:583–594.

Varmuza, K., and P. Filzmoser. 2016. Introduction to multivariate statistical analysis in chemometrics. CRC Press, Boca Raton, FL.

von Schilcher, F. 1976. The role of auditory stimuli in the courtship of *Drosophila melanogaster*. Animal Behaviour 24:18–26.

Waage, J. K. 1979. Reproductive character displacement in *Calopteryx* (*Odonata*: *calopterygidae*). Evolution 33:104–116.

Wagner, C. E., L. J. Harmon, and O. Seehausen. 2012. Ecological opportunity and sexual selection together predict adaptive radiation. Nature 487:366–369.

Walker, T. J. 1974. Character displacement and acoustic insects. American Zoologist 14:1137–1150.

Wang, R. L., and J. Hey. 1996. The speciation history of *Drosophila pseudoobscura* and close relatives: inferences from DNA sequence variation at the period locus. Genetics 144:1113–1126.

West-Eberhard, M. J. 1983. Sexual selection, social competition, and speciation. Quarterly Review of Biology 58:155–183.

Williams, M. A., A. G. Blouin, and M. A. Noor. 2001. Courtship songs of *Drosophila pseudoobscura* and *D. persimilis*. II. Genetics of species differences. Heredity 86:68–77.

Yamada, H., T. Sakai, M. Tomaru, M. Doi, M. Matsuda, and Y. Oguma. 2002. Search for species-specific mating signal in courtship songs of sympatric sibling species, *Drosophila ananassae* and *D. pallidosa*. Genes and Genetic Systems 77:97–106.

Yukilevich, R. 2012. Asymmetrical patterns of speciation uniquely support reinforcement in *Drosophila*. Evolution 66:1430–1446.

———. 2013. Tropics accelerate the evolution of hybrid male sterility in *Drosophila*. Evolution 67:1805–1814.

———. 2014. The rate test of speciation: estimating the likelihood of non-allopatric speciation from reproductive isolation rates in *Drosophila*. Evolution 68:1150–1162.

———. 2021. Data from: Reproductive character displacement drives diversification of male courtship songs in *Drosophila*. Dryad Digital Repository, American Naturalist, https://doi.org/10.5061/dryad.m63xsj41k.

Yukilevich, R., and F. Aoki. 2016. Is cascade reinforcement likely when sympatric and allopatric populations exchange migrants? Current Zoology 62:155–167.

Yukilevich, R., T. Harvey, S. Nguyen, J. Kehlbeck, and A. Park. 2016. The search for causal traits of speciation: divergent female mate preferences target male courtship song, not pheromones, in *Drosophila athabasca* species complex. Evolution 70:526–542.

Yukilevich, R., L. S. Maroja, K. Nguyen, S. Hussain, and P. Kumaran. 2018. Rapid sexual and genomic isolation in sympatric *Drosophila* without reproductive character displacement. Ecology and Evolution 8:2852–2867.

Yukilevich, R., and E. K. Peterson. 2019. The evolution of male and female mating preferences in *Drosophila* speciation. Evolution 73:1759–1773.

References Used for the Data

Alonso-Pimentel, H., H. G. Spangler, and W. B. Heed. 1995. Courtship sounds and behaviour of the two saguaro-breeding *Drosophila* and their relatives. Animal Behavior 50:1031–1039.

Asada, N., K. Fujiwara, H. Ikeda, and F. Hihara. 1992. Mating behavior in three species of the *Drosophila hypocausta* subgroup. Zoological Science 9:397–404.

Colegrave, N., H. Hollocher, K. Hinton, and M. G. Ritchie. 2000. The courtship song of African *Drosophila melanogaster*. Journal of Evolutionary Biology 13:143–150.

Costa, C. T. A., and F. M. Sene. 2002. Characterization of courtship sounds of species of the subgroup fasciola (*Diptera*, *Drosophilidae*, *Drosophila repleta* group): interspecific and interpopulational analyses. Brazilian Journal of Biology 62:573–583.

Cowling, D. E., and B. Burnet. 1981. Courtship songs and genetic control of their acoustic characteristics in sibling species of the *Drosophila melanogaster* subgroup. Animal Behaviour 29:924–935.

Crossley, S. A. 1986. Courtship-sounds and behaviour in the four species of the *Drosophila bipectinata* complex. Animal Behaviour 34:1146–1159.

Etges, W. J., K. F. Over, C. C. De Oliveira, and M. G. Ritchie. 2006. Inheritance of courtship song variation among geographically isolated populations of *Drosophila mojavensis*. Animal Behaviour 71:1205–1214.

Ewing, A. W., and J. A. Miyan. 1986. Sexual selection, sexual isolation and the evolution of song in the *Drosophila repleta* group of species. Animal Behaviour 34:421–429.

Hoikkala, A., and K. Y. Kaneshiro. 1997. Variation in male wing song characters in *Drosophila plantibia* (Hawaiian picture-winged *Drosophila* group). Journal of Insect Behavior 10:425–436.

Hoikkala, A., and J. Lumme. 1987. The genetic basis of evolution of the male courtship sounds in the *Drosophila virilis* group. Evolution 41:827–845.

Hoikkala, A., and P. Welbergen. 1995. Signals and responses of females and males in successful and unsuccessful courtships of three Hawaiian lek-mating *Drosophila* species. Animal Behaviour 50:177–190.

Ikeda, H., F. Hihara, N. Asada, K. Fujiwara, and F. J. Lin. 1983. Reproductive isolation among three species belonging to the *Drosophila hypocausta* subgroup. Pages 18–28 *in* Overseas scientific expedition for the collection of *Drosophilid* flies, 1971–1982.

Lakovaara, S., and A. Hoikkala. 1979. Analysis of the male courtship sounds of certain species of the *Drosophila virilis* group. Aquilo Series Zoologica 20:94–99.

Neems, R. M., K. Dooher, R. K. Butlin, and B. Shorrocks. 1997. Differences in male courtship song among the species of the *quinaria* group of *Drosophila*. Journal of Insect Behavior 10:237–246.

Noor, M. A., M. D. Schug, and C. F. Aquadro. 2000. Microsatellite variation in populations of *Drosophila pseudoobscura* and *Drosophila persimilis*. Genetics Research 75:25–35.

Noor, M. A., M. A. Williams, D. Alvarez, and M. Ruiz-García. 2000. Lack of evolutionary divergence in courtship songs of *Drosophila pseudoobscura* subspecies. Journal of Insect Behavior 13:255–262.

Tomaru, M., and Y. Oguma. 1994. Differences in courtship song in the species of the *Drosophila auraria* complex. Animal Behaviour 47:133–140.

Waldron, I. 1964. Courtship sound production in two sympatric sibling *Drosophila* species. Science 144:191–193.

Watson, E. T., E. Rodewald, and J. A. Coyne. 2007. The courtship song of *Drosophila santomea* and a comparison to its sister species *D. yakuba* (*Diptera*: *Drosophilidae*). European Journal of Entomology 104:145–148.

Associate Editor: Roger K. Butlin
Editor: Daniel I. Bolnick