TROPICS ACCELERATE THE EVOLUTION OF HYBRID MALE STERILITY IN *DROSOPHILA*

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Understanding the evolutionary mechanisms that facilitate speciation and explain global patterns of species diversity has remained a challenge for decades. The most general pattern of species biodiversity is the latitudinal gradient, whereby species richness increases toward the tropics. Although such a global pattern probably has a multitude of causes, recent attention has focused on the hypothesis that speciation and the evolution of reproductive isolation occur faster in the tropics. Here, I tested this prediction using a dataset on premating and postzygotic isolation between recently diverged *Drosophila* species. Results showed that while the evolution of premating isolation was not greater between tropical *Drosophila* relative to nontropical species, postzygotic isolation evolved faster in the tropics. In particular, hybrid male sterility was much greater among tropical *Drosophila* compared to nontropical species pairs of similar genetic age. Several testable explanations for the novel pattern are discussed, including greater role for sterility-inducing bacterial endosymbionts in the tropics and more intense sperm–sperm competition or sperm–egg sexual conflict in the tropics. The results imply that processes of speciation in the tropics may evolve at different rates or may even be somewhat different from those at higher latitudes.

KEY WORDS: Endosymbiosis, Haldane's rule, hybrid male sterility, latitudinal biodiversity gradient, patterns of speciation in *Drosophila*, postzygotic isolation, reproductive isolation.

Several studies have now used large-scale comparative approaches to test how reproductive isolation (henceforth abbreviated as "RI") evolves over time and how it is influenced by the biogeographic and ecological context. For instance, it has been shown that the evolution of prezygotic isolation is enhanced in sympatry (Dobzhansky et al. 1968; Coyne and Orr 1989, 1997; Noor 1997; Yukilevich 2012) and is correlated with the degree and direction of hybridization costs between sympatric species (Yukilevich 2012). It has also been shown that hybrid sterility typically evolves faster than hybrid inviability and that hybrid male sterility often precedes hybrid female sterility (Haldane 1922; Coyne and Orr 1989, 1997; Wu 1992; Price and Bouvier 2002). Finally, RI has been shown to correlate with the degree of ecological divergence (Funk et al. 2006) and the evolutionary rates of RI are known to vary substantially across different taxa (e.g., Sasa et al. 1998; Presgraves 2002; Mendelson 2003; Moyle et al. 2004). The discovery of these

patterns has provided a clear way to test theoretical predictions about speciation.

One of the most debated topics concerning rates and patterns of speciation revolves around the question of latitudinal biodiversity gradients found in many marine and terrestrial organisms (e.g., Ricklefs and Schluter 1993; Mittlebach et al. 2007; Schemske et al. 2009). Numerous hypotheses for explaining greater species richness in the tropics have been proposed, including those based on differences in rates of extinction and speciation between tropical and nontropical species (see Mittlebach et al. 2007). Although differences in extinction rates may be important (e.g., Weir and Schluter 2007), the question of whether rates of speciation and evolution of RI differ across latitudes is still largely unknown.

Many have proposed that mechanisms of speciation could ultimately differ with latitude. For instance, it has been argued

that tropical environments may promote greater genetic differentiation and allopatric speciation due to mountain ranges being more effective as barriers to dispersal (Janzen 1967; Moritz et al. 2000). At the same time, temperate species are more likely to experience repeated secondary contacts and recurrent gene flow due to cyclical glaciation events, limiting allopatric speciation (Dynesius and Jansson 2000). There is general support for greater genetic differentiation among tropical populations compared to nontropical populations (Martin and Mackay 2004). Others suggest that historically greater species richness in the tropics generates a positive feedback loop, further increasing ecological opportunity for specialization and niche divergence (e.g., Gentry 1989; Schemske 2002). Thus, biotic interactions and coevolutionary arms races are predicted to be more intense in the tropics, possibly accelerating rates of speciation as local populations have to adapt to dynamic biological communities (Dobzhansky 1950; Schemske 2002; Schemske et al. 2009). It has also been proposed that warmer temperatures of the tropics accelerate rates of biological processes, including faster molecular evolution and increased mutation rates (Rohde 1992; Martin and Palumbi 1993; Allen et al. 2006, but see Brohman and Cardillo 2003).

These potential differences between tropical and nontropical latitudes may translate to more rapid accumulation of RI in the tropics. This may include faster evolution of prezygotic isolation such as mating and habitat preferences and/or faster evolution of postzygotic isolation resulting from hybrid ecological inferiority, sterility, or inviability. A recent review of latitudinal biodiversity gradients has called attention to the fact that little has been done to explicitly test whether tropical species are evolving RI faster than their nontropical counterparts (Mittlebach et al. 2007). To date, the best dataset available to test this question is in the genus *Drosophila* (Coyne and Orr 1989, 1997; see recent extension in Yukilevich 2012).

In the present article, I performed such an analysis on species pairs of *Drosophila*. Although all informative species pairs were analyzed with respect to this question, the major goal was to ask whether RI evolves faster in the tropics particularly at the early stages of divergence. By restricting attention to this well-studied genus, the goal was to reach specific conclusions about latitudinal patterns of speciation in *Drosophila* in the hope that future work would test whether these findings also apply to other species groups.

Methods
THE DATA
In a recent paper describing new patterns of speciation in *Drosophila* (Yukilevich 2012), I extended earlier studies of Coyne and Orr (1989, 1997), and Bock (1984) by compiling published data on premating and postzygotic isolation indexes and their raw values for over 630 species pairs. Although much of the original indexes remained the same or similar, many were substantially updated. The total number of informative species pairs increased by sixfold (see Yukilevich 2012). Data on geographical range sizes and range overlap between species were also accumulated (for details, see below and Yukilevich 2012). The data, range maps, and relevant references are available at www.Drosophila-speciation-patterns.com. All analyses below were performed on raw and phylogenetically corrected datasets. Following Fitzpatrick and Turelli (2006) and Yukilevich (2012), the weighted phylogenetic correction was used by averaging non-independent values across phylogenetic nodes.

SPECIES GEOGRAPHICAL RANGE MAPS AND RANGE OVERLAP
Species range size maps from the literature were used to determine the absolute area for each species in square kilometers with Google Map Area Calculator: http://www.daftlogic.com/projectsgoogle-maps-area-calculator-tool.htm. Using the same approach, the absolute range size overlap between each species pair in square kilometers was determined. The percent of overlap for each species is equal to absolute range size overlap between the two species/absolute range size of that species. The average percent of geographical overlap between these species was then calculated by averaging these values (Yukilevich 2012).

PREMATING, POSTZYGOTIC, AND TOTAL ISOLATION INDEXES
Isolation indexes range from 0 (no isolation) to 1 (complete isolation). Premating isolation for each species pair was based on no-choice, single-choice, or multiple-choice mating experiments (the three choice tests were randomly distributed across species pairs). The data were based on percentage of successful copulations (inseminations) for each homotypic and heterotypic mating type during the period of the experiment. This was determined by either dissection of female reproductive tract to look for sperm or counting matings of each type during the experiment. The overall premating isolation index is equal to $1 - $ (sum of heterotypic matings%)/(sum of homotypic matings%).

Postzygotic isolation for each species pair was based on the percentage of F1 hybrid male and female sterility and inviability for each reciprocal heterotypic mating (see Yukilevich 2012). All hybrid sterility and inviability effects were considered. For each reciprocal mating, postzygotic isolation can range 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. Partial sterility and inviability of each hybrid sex was also accounted whenever

available by multiplying the above indexes by percentage of hybrid sterility or inviability. The overall postzygotic isolation index is the average of the reciprocal mating indexes.

Finally, Coyne and Orr (1989, 1997) and Ramsey et al. (2003) method was used to calculate total reproductive isolation (total RI) index, which determines the amount of gene flow species may be experiencing after each consecutive isolating barrier is accounted. This was done by first using the absolute individual component of premating isolation and then determining the extent to which the residual gene flow is prevented by postzygotic isolation. The total isolation is thus equal to: Premating Isolation $+$ ((1– Premating Isolation) $\times$ (Postzygotic Isolation)).

ESTIMATING LATITUDES AND ECOLOGICAL HABITATS OF SPECIES PAIR
Average latitude of species pairs
The latitude of each species was determined by using known range map of each species described above and identifying the GPS midpoint location of each species geographical range. The two latitudes for each species pair were then averaged.

Ecological habitats of species pairs
Because the mean latitude may not necessarily capture all the possible habitats that a species occupies, there may be instances where the mean latitude is in one category (e.g., tropical), but the species occupies habitats in multiple categories (e.g., both tropical and subtropical habitats). To address this issue, using the above range map of each species, I determined the ecological habitats (eco-zones) that each species occupies from a Global Forest Resource Assessment (GFRA) dataset on eco-zones, compiled by Food and Agriculture Organization of United Nations (FAO 2005). This was done by superimposing the geographical map of eco-zones over the species range map and then categorizing whether the species occupied any temperate, subtropical, and/or tropical habitats. Detailed information on occupied ecological zones of each species can be found at www.*Drosophila*-speciation-patterns.com. For the purposes of this article, each species was labeled as "tropical," "subtropical," or "temperate," or if it occupied a mixture of these habitats, it was labeled as "tropical-subtropical," "subtropical-temperate," or "all." No species occupied only a mixture of tropical and temperate habitats. Species pairs were then grouped into those that occupy temperate, subtropical, tropical, or a mixture of these habitats.

Results
THE GEOGRAPHY AND RI OF EARLY SPECIATION IN *DROSOPHILA*
The present study uncovered a total of 288 species pairs with known genetic distances and either complete information for pre-

mating isolation, postzygotic isolation, or both. As the interest is to understand whether RI evolves faster in the tropics compared to nontropics, although all informative species pairs were studied, detailed analyses were performed on pairs with low genetic distances, indicative of more recent divergence (as in Coyne and Orr 1989, 1997). Of the 288 informative cases, 112 species pairs had Nei's $D \leq 0.50$ and 57 species pairs had Nei's $D \leq 0.25$ (see Table S1). While a formal test of the relationship between range overlap and genetic distance was avoided (see Losos and Glor 2003), sympatry at early stages of divergence was not uncommon in *Drosophila* (Table S1). For instance, among the 57 species pairs with Nei's $D \leq 0.25$, 32 were sympatric (56%). Moreover, among these species pairs, the average percent of geographical overlap was 42% (Fitzpatrick and Turelli's [2006] method would increase this percentage even further). Similar results were found among species pairs with Nei's $D \leq 0.5$ (61 of 112 or 54% were sympatric).

Virtually, all of *Drosophila* species pairs with Nei's $D \leq 0.5$ showed incomplete premating and postzygotic isolation (Table S1). Considering all species pairs with Nei's $D \leq 0.5$ and both isolation indexes known, most cases showed incomplete total RI index: 40 of 42 (95%) allopatric species pairs and 34 of 39 (87%) sympatric pairs (see Methods for calculation). Below, I asked whether tropics have accelerated the evolution of RI at these earlier and later stages of divergence.

THE EFFECT OF TROPICS ON THE EVOLUTION OF PREMATING AND POSTZYGOTIC ISOLATION IN *DROSOPHILA*
Is the evolution of premating isolation different between tropical and nontropical taxa?
First, my analysis did not find any support for the hypothesis that the level of premating isolation is related to the average latitude between species pairs. For instance, using the raw data, no significant relationship between premating isolation and average latitude was found among younger and among older species pairs (species pairs with Nei's $D \leq 0.25$: $Rho_{Prem.Isol.} = -0.076$, $P = 0.60$, $n = 51$; pairs with Nei's $D \leq 0.5$: $Rho_{Prem.Isol.} = -0.06$, $P = 0.59$, $n = 98$; pairs with Nei's $D > 0.5$: $Rho_{Prem.Isol.} = -0.12$, $P = 0.13$, $n = 162$: also see Table 1). This relationship remained nonsignificant when species pairs were phylogenetically corrected ($Rho = -0.02$, $P = 0.87$), and when they were separated into allopatric and sympatric categories (see Table 1 for analysis on pairs with Nei's $D \leq 0.5$). Using ecological habitat information instead of average latitude also showed that species pairs living in the tropics did not show significantly greater premating isolation compared to species pairs living in either subtropical habitats, temperate habitats, or their combination (Kruskal–Wallis test on species pairs with Nei's $D \leq 0.5$: Prem. Isol.$_{tropical \times tropical} = 0.61$,

Table 1. The relationship between reproductive isolation and average latitude between *Drosophila* species.

Reproductive isolation	Rho (no. of pairs)	*P*-value	Adjusted *P*-value after Nei's *D* effect is removed	Bonferroni adjusted significance[1]
Premating isolation	−0.06 (98)	0.59	–	–
Postzygotic isolation	−0.41 (76)	0.0003	0.0005	Significant at $P < 0.05$
Allopatric species pairs				
Premating isolation	0.08 (44)	0.59	–	–
Postzygotic isolation	−0.46 (39)	0.003	0.005	Significant at $P < 0.05$
Sympatric species pairs				
Premating isolation	−0.01 (54)	0.92	–	–
Postzygotic isolation	−0.35 (37)	0.035	0.019	Not significant

Notes: Tests above are performed on phylogenetically uncorrected species pairs (see text for phylogenetically corrected results). All species pairs with Nei's $D \leq 0.5$ were considered. Adjusted *P*-values for partial correlation between reproductive isolation and the above variables are also shown after the effect of genetic distance (Nei's *D*) is removed (see text).

[1]Bonferroni adjusted *P*-value for significance = 0.0085.

Prem. Isol.$_{\text{temp/subtrop.} \times \text{temp/subtrop.}}$ = 0.63, Z-value = 0.41, $P = 0.68$). Indeed, it is clear that on average, tropical and nontropical species pairs both showed high levels of premating isolation (see Table S1). In total, this analysis did not support the hypothesis that premating isolation evolves faster in the tropics.

Is the evolution of postzygotic isolation different between tropical and nontropical taxa?

On the other hand, the relationship between the level of postzygotic isolation of species pairs and their average latitude was highly significant especially among younger species. Specifically, using the raw data, postzygotic isolation between species with Nei's $D \leq 0.5$ was found to be strongly negatively correlated with average latitude (see Table 1: $Rho_{\text{Postz. Isol.x lat.}} = -0.41$, $P = 0.0003$, $n = 76$). This relationship remained highly significant when the data were corrected for phylogenetic nonindependence ($Rho_{\text{phyl. corr.}} = -0.39$, $P = 0.0066$, $n = 48$). The presence of a few tropical mountain endemics had no effect on these results (data not shown). Upon closer inspection, the result is largely driven by the youngest species pairs with Nei's $D \leq 0.25$, for which the correlation between latitude and postzygotic isolation is strongest ($Rho = -0.57$, $P < 0.0001$, $n = 46$). Indeed, postzygotic isolation is not significantly related to latitude for older species pairs with Nei's $D > 0.5$ (data not shown).

Separating species pairs with Nei's $D \leq 0.5$ into allopatric and sympatric species pairs also showed highly significant negative correlations (see Table 1). However, allopatric species pairs exhibited a stronger relationship compared to sympatric taxa (Table 1). Moreover, when these statistics were corrected for multiple testing using Bonferroni adjustment, the test for sympatric species pairs was no longer significant, whereas tests for all species and for allopatric species remained significant (see Table 1). This indicates that accelerated evolution of postzygotic isolation in the tropics seems to especially occur among allopatric species.

Similar results were found when ecological habitat information was used to assess the question: Using raw data, species pairs with Nei's $D \leq 0.5$ that included one or both tropical species on average had significantly greater postzygotic isolation compared to species pairs that did not contain tropical species (either temperate, subtropical, or some combination of these habitats) (Kruskal–Wallis test on means: Postz.Isol.$_{\text{all pairs with tropical species}}$ = 0.39 ($n = 50$), Postz.Isol.$_{\text{all nontropical pairs (temp/subtrop.} \times \text{temp/subtrop.)}}$ = 0.092 ($n = 19$), Z-value = 3.9, $P < 0.0001$; phylog. corr. data: Z-value = 2.50, $P = 0.01$). Figure 1 plots postzygotic isolation as a function of genetic distance of species pairs and illustrates that the difference between tropical and nontropical taxa occurs at the earliest stages of divergence (this also occurs in phylogenetically corrected data: see Fig. S1).

Is the relationship between postzygotic isolation and latitude confounded by genetic distance?

Funk et al. (2006) demonstrated that the relationship between RI and ecological factors can be potentially confounded by genetic distance (age) of species. In the present case, greater postzygotic isolation in the tropics may simply be due to tropical taxa being on average older than nontropical taxa even among species pairs with Nei's $D \leq 0.5$. This would create a false impression that tropics per se are causing enhanced isolation. A test of this question revealed that there was no relationship between the average latitude of a species pair and its genetic distance for species pairs with Nei's $D \leq 0.5$ ($Rho_{\text{avg. latitude} \times \text{Nei's } D} = 0.028$, $P = 0.77$; phylog. corr. data: $Rho = 0.005$, $P = 0.97$). Moreover, when the effect of genetic distance was removed, the results still showed a highly
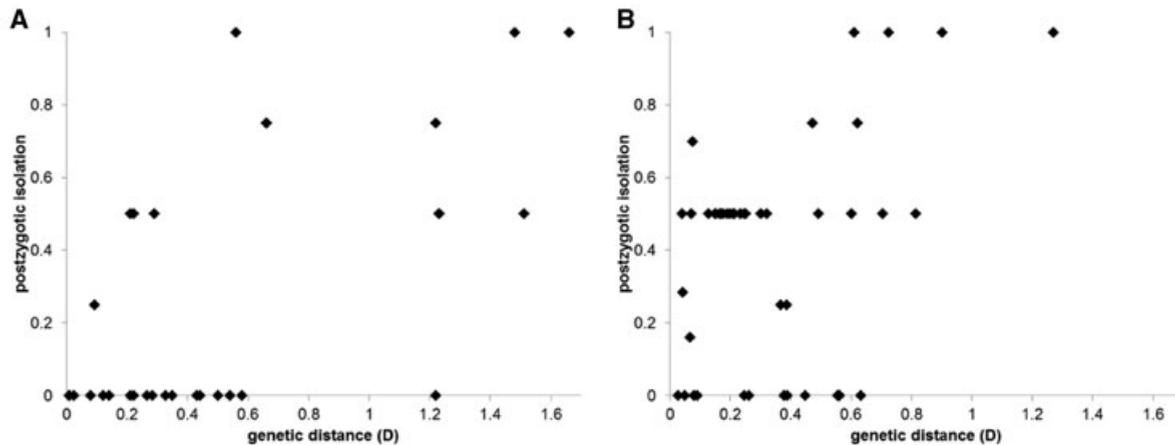
Figure 1. Relationship between postzygotic isolation and genetic distance (Nei's *D*) between: (A) temperate and/or subtropical species pairs and (B) tropical species pairs. Phylogenetically uncorrected data are shown to emphasize overall patterns (phylogenetically corrected data based on fewer points show same patterns: see Fig. S1). This information is based on inviability and sterility of F1 hybrids (see Table S1).

significant partial correlation between postzygotic isolation and average latitude (see Table 1). These results indicate that tropical taxa are not older than nontropical taxa and thus the relationship between postzygotic isolation and latitude is independent of genetic distance.

THE MECHANISM OF FASTER EVOLUTION OF POSTZYGOTIC ISOLATION IN THE TROPICS

The pattern described above can either be due to faster evolution of hybrid sterility or inviability effects in the tropics. Ecological and behavioral hybrid incompatibility is not included in these indexes because these data are still largely missing in *Drosophila*. Table 2 describes the nature of postzygotic isolation for 33 tropical species pairs and for 19 nontropical species pairs with Nei's $D \leq$ 0.5. These data illustrates that 24 of 33 (73%) tropical cases have some level of postzygotic isolation, compared to only four of 19 (21%) nontropical cases (*t*-test on proportions: *t*-value = 4.288, *P* < 0.0001; phylog. corr. data: tropical 15/19 (79%), non-tropical 4/14 (29%), *t*-value = 3.30, *P* = 0.0015). Interestingly, six of nine (67%) tropical species pairs with zero intrinsic postzygotic isolation are island endemics.

Of the 24 tropical species pairs with postzygotic isolation in Table 2, 22 cases (92%) showed exclusively hybrid male sterility, consistent with Haldane's rule (see Coyne and Orr 2004; the other two species pairs in addition to male sterility also showed some hybrid inviability). This finding is consistent with previous observations that F1 hybrid male sterility is usually the first postzygotic incompatibility to evolve in *Drosophila* hybrids (Coyne and Orr 1989; Wu 1992). Moreover, almost all of the tropical species pairs (20 of 22) had evolved hybrid male sterility in both reciprocal matings (Table 2). In contrast, temperate and subtropical species pairs

did not show a similar pattern (Table 2). Of the four nontropical species pairs with postzygotic isolation, only one exemplified exclusively hybrid male sterility. The other three cases showed both hybrid sexes evolving sterility and/or inviability and always in only one of the reciprocal crosses; the other cross of each species pair had viable and fertile hybrids.

These results lead to two major conclusions: First, the underlying difference in the level of postzygotic isolation between tropical versus nontropical species pairs is primarily driven by the differences in F1 hybrid male sterility. Second, these differences become even more striking when one compares the number of actual reciprocal crosses that show these hybrid effects in the tropics compared to nontropics.

Discussion

Arguably, one of the most generalizable patterns of biology is the observation that biodiversity increases toward the equator, known as the "latitudinal biodiversity gradient" (reviewed by Mittlebach et al. 2007). One of the hypotheses for this pattern is that species should accumulate RI faster in the tropics compared to more temperate climates. Here, I tested this prediction using a recently compiled dataset on premating and postzygotic isolation between *Drosophila* species.

THE NATURE OF EARLY SPECIATION IN *DROSOPHILA*

As the goal was to test whether tropical species evolve RI faster than nontropical species, a major focus was to determine if there were any differences in RI between species pairs at early stages of speciation. *Drosophila* species pairs at the early stages of speciation were equally likely to be geographically allopatric versus sympatric. It was also confirmed that the great majority of these

Table 2. Description of sterility and inviability effects of F1 hybrid offspring responsible for the differences in postzygotic isolation between tropical and nontropical *Drosophila*.

Sp1	Sp2	Habitat (Sp1)	Habitat (Sp2)	Percent sympatry	Genetic distance (Nei's D)	Premating isolation	Postzygotic isolation	Postzygotic hybrid fitness (cross 1 male, female /cross 2 male, female)
Tropical species pairs								
heteroneura	silvestris	Tropical	Tropical	0.83	0.03	0.80	0	Fertile, fertile/fertile, fertile
tropicalis tropicalis	tropicalis cubana	Tropical	Tropical	0	0.04	0.20	0.5	Sterile, fertile/sterile, fertile
paulistorum Centroamerican	paulistorum Transitional	Tropical	Tropical	0	0.04	0.13	0.29	61% sterile, fertile/53% sterile, fertile
silvestris W (Kona-side)	silvestris E (Hilo-side)	Tropical	Tropical	0	0.05	0.29	0	Fertile, fertile/fertile, fertile
paulistorum Andean-Brazilian	paulistorum Transitional	Tropical	Tropical	0.06	0.07	0.56	0.16	32% sterile, fertile/32% sterile, fertile
paulistorum Andean-Brazilian	paulistorum Centroamerican	Tropical	Tropical	0.03	0.07	0.68	0.5	Sterile, fertile/sterile, fertile
paulistorum Interior	paulistorum Orinocan	Tropical	Tropical	0	0.07	0.41	0.7	sterile, fertile (part. inviable)/sterile, fertile (part. inviable)
melanogaster West Africa/Caribbean	melanogaster South Africa (Zimb.)	Tropical	Tropical	0	0.08	0.27	0	Fertile, fertile/fertile, fertile
ananassae	pallidosa	Tropical	Tropical	0.11	0.09	0.85	0	Fertile, fertile/fertile, fertile
paulistorum Centroamerican	paulistorum Interior	Tropical	Tropical	0	0.13	0.12	0.5	Sterile, fertile/sterile, fertile
paulistorum Interior	paulistorum Transitional	Tropical	Tropical	0	0.15	0.19	0.5	Sterile, fertile/sterile, fertile
paulistorum Amazonian	paulistorum Centroamerican	Tropical	Tropical	0.04	0.15	0.95	0.5	Sterile, fertile/sterile, fertile
paulistorum Andean-Brazilian	paulistorum Orinocan	Tropical	Tropical	0.26	0.17	0.86	0.5	Sterile, fertile/sterile, fertile
paulistorum Amazonian	paulistorum Andean-Brazilian	Tropical	Tropical	0.56	0.17	0.86	0.5	Sterile, fertile/sterile, fertile
paulistorum Amazonian	paulistorum Transitional	Tropical	Tropical	0.55	0.18	1	0.5	Sterile, fertile/sterile, fertile
paulistorum Amazonian	paulistorum Orinocan	Tropical	Tropical	0.67	0.19	1	0.5	Sterile, fertile/sterile, fertile
paulistorum Orinocan	paulistorum Transitional	Tropical	Tropical	0.36	0.20	0.38	0.5	Sterile, fertile/sterile, fertile
paulistorum Centroamerican	paulistorum Orinocan	Tropical	Tropical	0.04	0.21	0.14	0.5	Sterile, fertile/sterile, fertile
heteroneura	planitibia	Tropical	Tropical	0	0.21	0.59	0.5	Sterile, fertile/sterile, fertile
paulistorum	pavlovskiana	Tropical	Tropical	0.50	0.23	0.94	0.5	Sterile, fertile/sterile, fertile
equinoxialis caribbeansis	equinoxialis equioxialis	Tropical	Tropical	0	0.25	0.02	0.5	Sterile, fertile/sterile, fertile
differens	heteroneura	Tropical	Tropical	0	0.25	0.53	0	Fertile, fertile/fertile, fertile
planitibia	silvestris	Tropical	Tropical	0	0.25	0.42	0.5	Sterile, fertile/sterile, fertile
differens	silvestris	Tropical	Tropical	0	0.26	0.75	0	Fertile, fertile/fertile, fertile
yacuba	santomea	Tropical	Tropical	0.29	0.3	0.84	0.5	Sterile, fertile/sterile, fertile (& Fecund. Diff. 2nd cross ≪)
mauritiana	sechellia	Tropical	Tropical	0	0.32	0.92	0.5	Sterile, fertile/sterile, fertile
pennae	leontia	Tropical	Tropical	0	0.37	0.98	0.25	Sterile, fertile/sterile, fertile
sulfurigaster albostrigata	sulfurigaster sulfurigaster	Tropical	Tropical	0	0.38	0.13	0	Fertile, fertile/fertile, fertile

(Continued)

Table 2. Continued.

Sp1	Sp2	Habitat (Sp1)	Habitat (Sp2)	Percent sympatry	Genetic distance (Nei's D)	Premating isolation	Postzygotic isolation	Postzygotic hybrid fitness (cross 1 male, female /cross 2 male, female)
sulfurigaster albostrigata	*sulfurigaster bilimbata*	Tropical	Tropical	0	0.39	0.43	0.25	Sterile, fertile/fertile, fertile
sulfurigaster albostrigata	*pulaua*	Tropical	Tropical	0.58	0.39	0.99	0	Fertile, fertile/fertile, fertile
sulfurigaster bilimbata	*pulaua*	Tropical	Tropical	0	0.45	0.92	0	Fertile, fertile/fertile, fertile
starmeri	*venezolana*	Tropical	Tropical	1	0.47	0.79	0.75	Larvae only/sterile, fertile
sulfurigaster bilimbata	*sulfurigaster neonasuta*	Tropical	Tropical	0	0.49	0.60	0.5	Fertile or sterile (see references), fertile/sterile, fertile
Temperate and subtropical species pairs								
americana	*texana*	Temperate	Subtropical	0.36	0.01	0.06	0	Fertile, fertile/fertile, fertile
americana western (brown morph)	*americana eastern (black morph)*	Temperate	Temperate	0.09	0.01	0.22	0	Fertile, fertile/fertile, fertile
athabasca EA	*athabasca EB*	Temperate	Temperate	0.53	0.02	0.92	0	Fertile, fertile/fertile, fertile
hubeiensis	*sinobscura*	Subtropical	Subtropical	0	0.08	0.09	0	Fertile, fertile/fertile, fertile
triauraria	*quadraria*	Temperate-subtropical	Subtropical	0	0.09	0.09	0.25	Fertile, fertile/sterile, fertile
athabasca WN	*athabasca EA*	Temperate	Temperate	0.13	0.12	0.94	0	Fertile, fertile/fertile, fertile
athabasca WN	*athabasca EB*	Temperate	Temperate	0	0.14	0.35	0	Fertile, fertile/fertile, fertile
borealis	*montana*	Temperate	Temperate-subtropical	0.58	0.21	0.99	0.5	Fertile, fertile/inviable, sterile
lacicola	*montana*	Temperate	Temperate-subtropical	0.09	0.21	0.95	0	Fertile, fertile/fertile, fertile
biauraria	*triauraria*	Temperate-subtropical	Temperate-subtropical	0.98	0.22	0.59	0	Fertile, fertile/fertile, fertile
auraria	*triauraria*	Temperate-subtropical	Temperate-subtropical	0.71	0.22	0.80	0.5	Inviable, inviable/fertile, fertile
auraria	*biauraria*	Temperate-subtropical	Temperate-subtropical	0.70	0.27	0.84	0	Fertile, fertile/fertile, fertile
biauraria	*quadraria*	Temperate-subtropical	Subtropical	0	0.28	0.43	0	Fertile, fertile/fertile, fertile
flavomontana	*montana*	Temperate	Temperate-subtropical	0.54	0.29	0.99	0.5	Fertile, fertile/sterile, sterile
bifasciata Europe	*bifasciata Japan*	Temperate	Temperate-subtropical	0	0.33	–	0	Fertile, fertile/fertile, fertile
lummei	*virilis*	Temperate	Subtropical	0.79	0.35	0.26	0	Fertile, fertile/fertile, fertile
americana	*novamexicana*	Temperate	Temperate-subtropical	0	0.43	0.43	0	Fertile, fertile/fertile, fertile
novamexicana	*texana*	Temperate-subtropical	Subtropical	0	0.44	0.41	0	Fertile, fertile/fertile, fertile
novamexicana	*virilis*	Temperate-subtropical	Subtropical	0	0.5	0.51	0	Fertile, fertile/fertile, fertile

Notes: Only species pairs with Nei's $D \leq 0.5$ are described. Phylogenetically uncorrected data are shown to avoid averaging sterility and inviability effects of hybrids across multiple species pairs. The *paulistorum* species complex is overrepresented in the tropics, but even when this data are corrected for phylogenetic nonindependence, the difference in postzygotic isolation between tropics and nontropics remains highly significant (see text).

young species pairs had not yet accumulated complete premating, postzygotic, or total RI, even in sympatry. Either this latter finding means that our laboratory assays of RI do not accurately capture true RI between sympatric taxa (e.g., see Coyne and Orr 2004, p. 75) or that sympatric *Drosophila* experience some gene flow. Consistent with the latter hypothesis, Nosil (2012) used the *Drosophila* dataset to show that premating isolation tends to be weaker between species pairs with the highest levels of sympatry. Although alternative explanations for that pattern are possible, it was argued that this may be a result of gene flow impeding speciation at the highest levels of sympatry (Nosil 2012). Both findings suggest that future work should aim to empirically determine rates of gene flow between sympatric *Drosophila* species.

EVIDENCE FOR FASTER EVOLUTION OF POSTZYGOTIC ISOLATION, BUT NOT PREMATING ISOLATION IN THE TROPICS

My results further revealed novel patterns of speciation related to latitude in *Drosophila*. First, there was no evidence that premating isolation evolved faster in the tropics. These results were seen regardless of whether comparisons used species latitudes or species ecological habitat information. These findings imply that processes associated with the evolution of mating preferences (e.g., the intensity of sexual selection or reinforcement speciation) do not appear to differ across latitude in *Drosophila*.

However, there was strong support for faster accumulation of postzygotic isolation between tropical species pairs compared to nontropical pairs. This finding was significant at early stages of divergence (Nei's $D \leq 0.5$), and was most pronounced for species pairs with Nei's $D \leq 0.25$. The result was robust to the data being corrected for phylogenetic nonindependence as well as for multiple testing, and when the effect of genetic distance was removed. Only when sympatric pairs were analyzed separately from allopatric pairs and corrected for multiple testing, did the sympatric group fail to show a significant relationship. One explanation for this is that the evolution of postzygotic isolation likely requires a substantial allopatric phase. Therefore, the rapid accumulation of postzygotic isolation in the tropics is expected to be particularly acute among allopatric taxa. Ascertainment bias may also have occurred, such that sympatric taxa in the tropics that had stronger postzygotic isolation without significant prezygotic isolation may have gone extinct before they could be studied (e.g., Paterson 1978; Templeton 1981).

FASTER EVOLUTION OF HYBRID MALE STERILITY IN THE TROPICS IS THE PRIMARY MECHANISM FOR LATITUDINAL PATTERN

Inspection of the data revealed that the relationship between postzygotic isolation and latitude is primarily driven by faster evolution of F1 hybrid male sterility between tropical species compared to nontropical species. Nearly all cases of early postzygotic isolation in the tropics showed exclusively hybrid male sterility, whereas the few nontropical taxa with evidence of postzygotic isolation largely showed inviability and sterility effects in both sexes. Although the small number of data points with postzygotic isolation in the nontropics precludes one from formally testing whether these are meaningful differences in the type of postzygotic isolation that evolves between tropical versus nontropical species, future work may be able to test this intriguing possibility. Below, I describe several possible explanations for why hybrid male sterility may evolve faster in the tropics compared to nontropics.

POSSIBLE CAUSES FOR FASTER EVOLUTION OF HYBRID MALE STERILITY IN THE TROPICS

Any explanation for faster hybrid male sterility in the tropics must consider that premating isolation did not evolve faster in the tropics compared to nontropics and that the differences I observed were not due to greater genetic distance between tropical species pairs. Therefore, general differences in generation times, population sizes, gene flow, or other demographic processes across latitudes are unlikely to explain the observed pattern (see Mittlebach et al. 2007). For instance, tropical species are typically assumed to experience more generations per year compared to nontropical taxa (Mittlebach et al. 2007). But because I have explicitly compared species pairs with similar genetic distances, the pattern cannot be explained by the relative age of the species pairs. Similar arguments can be made against other demographic differences that in principle can increase the genetic distance between tropical taxa compared to nontropical taxa. It appears that specific processes associated with the evolution of spermatogenesis may be driving these differences.

One possible explanation for faster hybrid male sterility in the tropics is the role of bacterial endosymbionts in *Drosophila*. Most of the evidence for these nongenetic, but heritable agents of hybrid male sterility comes from work done on *Drosophila paulistorum* semispecies, which interestingly, make up a substantial proportion of the present cases of faster hybrid male sterility in the tropics (see Table 2; reviewed by Ehrman et al. 1995). Ehrman and others have shown that F1 hybrid male sterility between different races of this complex is often caused by different endosymbiotic subspecies of *Streptococcus faecalis* that disturb hybrid male spermatogenesis (Dobzhansky and Pavlovsky 1966; Somerson et al. 1984). Faster hybrid male sterility in the tropics can be explained if these male-sterility-inducing bacterial endosymbionts show latitudinal biodiversity gradients. Under this scenario, tropical *Drosophila* would be more likely to acquire different types of incompatible endosymbionts compared to nontropical species. This hypothesis

may also explain why most tropical species pairs that did not evolve hybrid male sterility were island endemics; one may speculate that *Drosophila* endosymbiotic bacteria may be less common on oceanic islands. Finally, this explanation is consistent with the idea that speciation in the tropics is a result of coevolutionary and biotic interactions with other species (Schemske et al. 2009).

Other possible explanations for enhanced male sterility in the tropics could be related to more intense sperm competition for egg fertilization among males ("sperm precedence"; Clark et al. 1995, 1999; reviewed in Swanson and Vacquier 2002) or more intense male–female sexual conflict at the sperm–egg surface protein level in the tropics (Rice 1996; Eberhard 1996; Partridge and Parker 1999; Swanson and Vacquier 2002). For instance, if competition for egg fertilization among males is more intense in the tropics compared to nontropics, genes specifically involved with spermatogenesis may be diverging at higher rates between tropical species compared to nontropical species. Thus, as a byproduct, hybrid spermatogenesis is more likely to be disrupted and result in higher levels of hybrid male sterility once the two species are crossed in the laboratory. These explanations predict that sperm–sperm competition and/or sperm–egg protein interactions are more intense among tropical *Drosophila*.

Finally, it is possible that higher temperatures of the tropics may accelerate spermatogenesis evolution and thus lead to faster hybrid male sterility between species. This hypothesis is consistent with the claim that spermatogenesis is particularly sensitive to external conditions (Wu and Davis 1993). For instance, it is possible that spermatogenesis may be exposed to greater number of mutations than other tissues, causing faster genetic divergence between sperm of tropical species, which could lead to faster hybrid male sterility in the tropics. Additional work is required to determine if these or other explanations for faster hybrid male sterility in the tropics are correct.

IMPLICATIONS FOR LATITUDINAL BIODIVERSITY GRADIENTS

What does faster hybrid male sterility in the tropics imply about latitudinal biodiversity gradients and rates of speciation? For *Drosophila* it possibly suggests that rates of speciation may be higher in the tropics as a result of accumulating postzygotic isolation faster. Although faster hybrid male sterility in the tropics is most evident in allopatry, it is also a factor in sympatry (see above). This can prevent gene flow between incipient species directly, and/or cause prezygotic isolation to evolve faster due to reinforcement (e.g., Coyne and Orr 2004; Yukilevich 2012). Both processes will facilitate the process of speciation and contribute to the latitudinal biodiversity gradient.

In summary, this article characterizes a novel pattern of speciation in *Drosophila*, showing that postzygotic isolation is greater among recently diverged tropical species compared to nontropical species. As far as the author knows, this is the first time that the evolution of a reproductive isolating barrier has been shown to differ across latitudes in any group of organisms. The pattern is primarily driven by faster evolution of hybrid male sterility in the tropics, consistent with Haldane's rule in *Drosophila*. Several possible hypotheses for the observed pattern are discussed. Further work is necessary to test these possible explanations and to determine whether similar relationships between RI and latitude occur in other taxa.

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Supporting Information

Additional Supporting Information may be found in the online version of this article at the publisher's website:

Table S1. Excel spreadsheet of *Drosophila* species and their reproductive and biogeographical information.

Figure S1. Relationship between postzygotic isolation and genetic distance for: (A) temperate and/or subtropical species pairs and (B) tropical species pairs.