INCIPIENT SEXUAL ISOLATION AMONG COSMOPOLITAN *DROSOPHILA MELANOGASTER* POPULATIONS

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Understanding the biological conditions and the genetic basis of early stages of sexual isolation and speciation is an outstanding question in evolutionary biology. It is unclear how much genetic and phenotypic variation for mating preferences and their phenotypic cues is segregating within widespread and human-commensal species in nature. A recent case of incipient sexual isolation between Zimbabwe and cosmopolitan populations of the human-commensal fruit fly *Drosophila melanogaster* indicates that such species may initiate the process of sexual isolation. However, it is still unknown whether other geographical populations have undergone evolution of mating preferences. In this study we present new data on multiple-choice mating tests revealing partial sexual isolation between the United States and Caribbean populations. We relate our findings to African populations, showing that Caribbean flies are partially sexually isolated from Zimbabwe flies, but mate randomly with West African flies, which also show partial sexual isolation from the United States and Zimbabwe flies. Thus, Caribbean and West African populations seem to exhibit distinct mating preferences relative to populations in the United States and in Zimbabwe. These results suggest that widespread and human-commensal species may harbor different types of mating preferences across their geographical ranges.

KEY WORDS: Genetic variation, incipient speciation, mating behavior, range expansion, rapid evolution, reproductive isolation, sexual selection.

The evolution of reproductive isolation and speciation has recently received a great deal of both theoretical and empirical attention (for reviews see Coyne and Orr 2004; Gavrilets 2004). One of the most pervasive conclusions of this body of research has been the idea that speciation becomes more probable in allopatry and is in general more likely with increasing geographical distance between populations (e.g., Mayr 1963; Coyne and Orr 2004). Geographically distant populations are more likely to experience both divergent ecological selective conditions as well as a reduction in homogenizing gene flow that is likely to be one of the greatest impediments to the evolution of isolating mechanisms (e.g., Dobzhansky 1937; Mayr 1942, 1963; Coyne and Orr 2004; Gavrilets 2004). However, many questions still remain about the exact biological conditions and the genetic basis of incipient reproductive isolation.

Of particular interest are geographically widespread species that have attained this status fairly recently either due to natural causes or with the aid of human transport (Dobzhansky 1965, 1973; Mayr 1965; Parsons 1983). It is well known that such widespread species often show clinal geographical differentiation, but what is less clear is the extent to which these species segregate genetic and phenotypic variation for incipient sexual and reproductive isolation (i.e., clinal sexual isolation; Mayr 1963; Endler 1977; Lande 1982; Doebeli and Dieckmann 2003). In principle, the very processes responsible for how these species become geographically widespread, namely high migration rates and rapid colonization of novel environments, should generally prevent populations of these species from diverging. On the other hand, because these species often span great geographical distances, often experiencing a wide range of environmental

and ecological conditions, there is potential to diverge in phenotypic and behavioral traits that may lead to incipient sexual isolation.

It is still unclear which factors are generally more important in the evolution of widespread species. This question becomes especially pertinent when considering recently widespread human commensals, because we would expect that migration and gene flow is still ongoing and is relatively high in these species compared to species that spread due to natural processes (e.g., Carson 1965; Dobzhansky 1965; Parsons 1983; Coyne and Orr 2004). Thus it is interesting to ask whether there is variation for mating preference behaviors within such species and whether there is potential for early stages of sexual isolation as in other systems of recently diverged populations or species (e.g., Verrell and Arnold 1989; Tilley et al. 1990; Endler and Houde 1995; Johannesson et al. 1995; Noor 1995; Funk 1998; Tregenza et al. 2000; Jiggins et al. 2001; Nosil et al. 2002; Pfennig and Simovich 2002; Tregenza 2002; Jiggins et al. 2004; Boughman et al. 2005; Ortíz-Barrientos and Noor 2005).

One of the few and perhaps best known cases of incipient sexual isolation in a recently widespread human-commensal species is the fruit fly, *Drosophila melanogaster* (Wu et al. 1995). This species has expanded from its ancestral African range to encompass most of the world in the last several thousand to hundred years with the aid of human transport (David and Capy 1988; Lachaise et al. 1988; Lachaise and Silvain 2004; Keller 2007). Despite earlier claims that this species mates randomly across its range (Henderson and Lambert 1982), recent evidence indicates that some African populations in and near Zimbabwe ("Z-type") have evolved incipient sexual isolation from "cosmopolitan" ("M-type") populations (Wu et al. 1995; Hollocher et al. 1997a,b; Greenberg et al. 2003).

Females from both Zimbabwe and cosmopolitan strains tend to preferentially mate with their own "Z-type" or "M-type" males, respectively, with some Zimbabwe strains showing very strong mating preferences (Wu et al. 1995; Hollocher et al. 1997a). The Zimbabwe–cosmopolitan sexual isolation also parallels substantial divergence in nuclear genes (Begun and Aquadro 1993), microsatellite loci (Kauer et al. 2002; Caracristi and Schlotterer 2003; Kauer and Schlotterer 2004), chromosomal inversions (Aulard et al. 2002), and various phenotypic traits, including body size, pigmentation, cuticular hydrocarbon composition, and wing beat frequency, that are all known to be under genetic control (David and Capy 1988; Colegrave et al. 2000; Rouault et al. 2001; Takahashi et al. 2001). This system suggests that the evolution of reproductive isolation in recently widespread and human-commensal species is possible.

However, it is still unclear whether other populations in this species may also show divergence in mating behaviors and

preferences. With the possible exception of "microhabitat" isolation in Brazzaville populations of West Africa (Capy et al. 2000; Haerty et al. 2002, 2005), and segregation of weaker Zimbabwe-like mating preferences across South Africa (Hollocher et al. 1997a), no other large-scale geographic case of sexual isolation has been described in this species (Henderson and Lambert 1982). In general, the cosmopolitan populations of *D. melanogaster* are assumed to mate randomly with one another and are not known to segregate genetic and phenotypic variation for sexual isolation (Handerson and Lambert 1982; Hollocher et al. 1997a).

However, previous authors have noted that Caribbean island *D. melanogaster* populations show some peculiar phenotypic and pheromonal differences from other cosmopolitan populations (David and Capy 1988). Of especial interest is the fact that Caribbean populations are the only populations outside of Africa known to harbor the African insertion allele at the *desaturase* locus (Takahashi et al. 2001). This locus solely determines a female cuticular hydrocarbon polymorphism between African and non-African flies and has been recently implicated in the sexual isolation between Zimbabwe and cosmopolitan strains (Greenberg et al. 2003; but see Coyne and Elwyn 2006). Further, Caribbean populations are known to segregate exceptional African-like morphology, making them phenotypically distinct from other cosmopolitan populations in the United States and Europe (Capy et al. 1993, 1994). Recent microsatellite evidence also indicates that United States flies are more genetically similar to African flies than are European flies, suggesting that African alleles may have introgressed into North America, possibly via the Caribbean islands (Caracristi and Schlotterer 2003, but see Capy et al. 1986).

Despite these intriguing phenotypic and genetic observations, the Caribbean populations remain largely unexplored. So far, mating preferences of only four isofemale lines have been studied. These lines were more similar to cosmopolitan than Zimbabwe lines (Fang et al. 2002). In the present work we remedy this situation by explicitly performing multiple-choice mating tests across southeastern United States and Caribbean populations. Our findings reveal that some Caribbean populations exhibit partial sexual isolation from United States populations and that there is a substantial geographical variation in the presence and strength of these mating preferences among the islands. Further, we find that Caribbean flies mate randomly with West African flies and flies from both of these regions show partial sexual isolation from United States and Zimbabwe populations. These results suggest that *D. melanogaster* cosmopolitan populations are segregating mating preference behaviors that may be distinct from those of Zimbabwe lines. This implies that widespread and human-commensal species may possibly evolve different types of mating preferences across their ranges.

Materials and Methods

STUDY SYSTEM AND REARING CONDITIONS OF ISOFEMALE LINES

In the summer of 2004, R. Yukilevich collected wild females (established as isofemale lines) from 37 locations, 18 from the southeastern United States (Missisipi, Alabama, Georgia, and Florida) and 19 from the islands of the Bahamas. Additional collections were undertaken in 2005 and 2006 in St. Lucia and Haiti (Fig. 1). African strains were acquired from J. Pool and C. Aquadro in 2005. These included 31 isofemale lines from West Cameroon (Mbalang-Djalingo) collected by J. Pool in 2004, 13 isofemale lines collected in 1990 from Lake Sengwa, Zimbabwe (Zim-s) that were previously studied by Wu et al. (1995) and Hollocher et al. (1997a), and 20 isofemale lines collected in 1994 from Lake Kariba, Zimbabwe (Zim-k). The two Zimbabwe locations are about 50 km apart. Prior to our experiments, all isofemale lines were maintained in the laboratory at Stony Brook University on instant *Drosophila* food (Carolina Biol. Supply Inc., Burlington, NC) in a 25°C incubator with a 12h light:12h dark regime. Thus, other than for the Zimbabwe lines, our analyses were performed on lines that were less than two-year old.
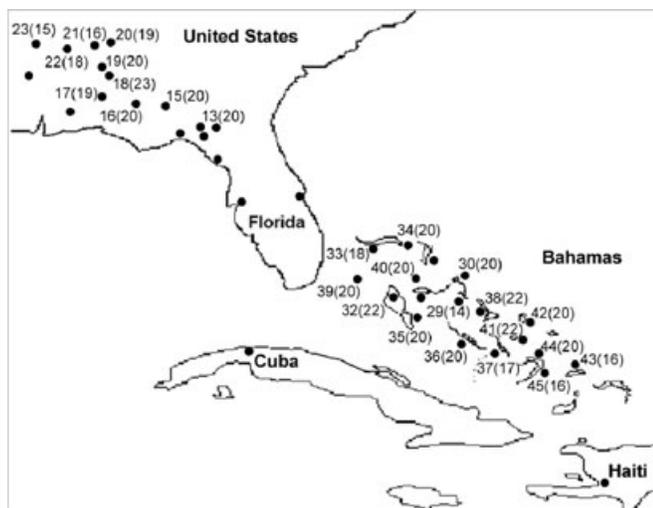
MULTIPLE-CHOICE MATING TESTS

All mating trials were done in the laboratory at Stony Brook University in 2005 and 2006. We followed the recommendation of Cesares et al. (1998) by concentrating on multiple-choice mating trials because mating propensity and preference are known to be

Figure 1. Map of localities in southern United States and Bahamas used in multiple-choice mating tests of sexual isolation. Each locality is represented by an identification number, followed by the number of isofemale lines (in parentheses) from each location used in mating tests. Other localities in which lines are available, but were not tested for mate choice are shown as isolated dots.

confounded in no-choice male and female tests (see also Coyne et al. 2005). We also studied a single US-Bahamas comparison under no-choice mating conditions that qualitatively confirmed our multiple-choice results. We randomly chose two locations at a time. For multiple-choice mating tests we used individuals from all available isofemale lines from each location (see Fig. 1). For each pairwise test we simultaneously set up all available isofemale lines of both locations by placing seven fertilized females into each experimental narrow glass vial (25 × 95 mm) and allowed them to lay eggs for four days under identical conditions. We then cleared the vials, inserted paper for pupation, and waited about 15 days until eclosion of adults. Virgin males and females from each line were then collected using light CO_2 anesthesia and were aged separately in pooled sex-specific vials for four to five days. Individuals of each line were equally represented from each location. The day before the mating trial, we randomly picked equal numbers of females and males and placed them separately on instant *Drosophila* colored food for about 12 h to mark their abdomens for identification (color has no effect on mating preferences; Wu et al. 1995; Boake et al. 2003).

We performed all mating trials within three to 5 h of "lights on" in the laboratory. We used a common Plexiglas mating chamber (28_L × 17.5_H × 16_W cm) by placing standard corn meal/molasses/agar fly food on the floor and walls of the chamber to simulate *D. melanogaster* mating in the wild, which typically takes place on or near food. For each replicate, we introduced 30 males from each location into the chamber without anesthesia by simply opening the vial into a hole in the chamber and letting the individuals fly out. These individuals were allowed to habituate for 5 min. We then introduced 30 females from each location using the same technique, for a total of 120 individuals per replicate test. We let the mating trials run until 50% of possible copulations had occurred (as recommended by Cesares et al. 1998 to avoid bias) and then placed the chamber into a −20°C or −80°C freezer for about 20 min to kill the flies in the state of copulation (as in Boake et al. 2003). This procedure avoids disturbing copulating pairs and is effective at retrieving about 98–100% of copulations (Boake et al. 2003). We then scored all copulations based on abdomen color. We cleaned the chambers between tests with 95% ethanol, followed by soap and water to ensure all pheromones from previous tests had been eliminated. Note that all replicates used the same isofemale lines.

The approach of using all isofemale lines from each locality to perform mating tests has several advantages over strategies that focus on testing specific isofemale lines (as in Wu et al. 1995; Hollocher et al. 1997a). The first advantage is that we can survey the mating behavior of many isofemale lines simultaneously in a given experiment. This is more likely to mimic mating choices in the wild because it is very likely that individuals encounter potential mates with different genetic backgrounds during mating.

Second, introducing individuals from all isofemale lines of a given population into the experimental mating cage avoids any biases that may come from "vial-effects" or individuals having unusual preferences for members of the same isofemale line. An individual is able to choose a mating partner from many isofemale lines of its native and foreign location. This approach also allows the important possibility of behavioral interactions among individuals of different isofemale lines. Therefore this design is a better representation of a "populational" measure of sexual isolation as opposed to an "isofemale" line measure that may or may not be representative of mating behavior of the population as a whole. Because we are interested in surveying many populations for sexual isolation, it would have been prohibitively difficult to perform many pairwise isofemale line tests for any sensible number of population pairs. The major disadvantage of this approach is that we cannot identify specific isofemale lines that show especially strong sexual isolation as has been done by Wu et al. (1995) and Hollocher et al. (1997a,b). This is important for genetic dissection of mating behavior, which is not the focus of the present work.

We used the sexual isolation index I_{psi} of Rolán-Álvarez and Caballero (2000), which ranges from -1 to $+1$ with $0 =$ random mating. The standard joint sexual isolation index of Merrel (1950; see also Malagolowkin-Cohen et al. 1965) gave virtually identical results. Significance and P-values were derived by resampling 10,000 times in *JMATING* software (http://webs.uvigo.es/acraaj/JMsoft.htm – Carvajal-Rodríguez and Rolán-Álvarez 2006). We also performed a χ^2 contingency test of independence, which tests if there is a significant mating interaction between individuals of two locations (Sokal and Rohlf 1995).

Results

INCIPIENT SEXUAL ISOLATION BETWEEN UNITED STATES AND CARIBBEAN FLIES

Table 1 shows the results of pairwise multiple-choice tests of sexual isolation performed between different localities both within southeastern United States and Bahamas and between these regions. First, among the 11 different pairwise tests within regions, none showed a significant excess of homotypic matings or deviation from random mating, as indicated by extremely low sexual isolation (I_{psi}) indexes and chi-square values. One test yielded a fairly high negative I_{psi} index between two neighboring United States localities, but was not significant. There was no significant heterogeneity within either region among different pairwise tests ($G_{Sex.Isol.Heterog.(withinUS)} = 2.61$; df $= 8$; $P = 0.95$; $G_{Sex.Isol.Heterog.(withinBahamas)} = 0.56$; df $= 10$; $P > 0.995$). After a total of 1629 copulations the average sexual isolation indexes for within United States and within-Bahamas did not significantly deviate from random mating

(average $I_{psi(US)} = 0.0019$ (SD $= 0.032$), $\chi^2 = 0.379$, $P = 0.544$; average $I_{psi(Bahamas)} = -0.001$ (SD $= 0.029$), $\chi^2 = 0.0006$, $P = 0.973$; Fig. 2A). Together these results show that there does not appear to be geographically based mating preference within either the United States or the Bahamas.

However, tests performed between United States and Bahamas populations gave strikingly different results. For a total of 18 United States and Bahamas pairwise tests performed, the average I_{psi} index was 0.0896 with a standard deviation of 0.017, which is significantly different from random mating (3785 copulations, $\chi^2 = 29.27$, $P < 10^{-5}$). This means on average 54% homotypic matings relative to 0.46% heterotypic matings. This index is also significantly different from average I_{psi} indexes of within United States and within-Bahamas comparisons ($P < 0.017$; Fig. 2A). We found variation in the presence and strength of sexual isolation among different United States and Bahamas pairwise tests. Out of a total of 18 tests, seven had significant chi-square values with an average of 58% homotypic and 42% heterotypic matings and another test had marginal significance with 56% homotypic and 44% heterotypic matings. Thus, nearly half of the United States Bahamas pairwise tests revealed significant assortative mating. For these eight pairwise tests, the average I_{psi} index equals 0.165 with a standard deviation of 0.026 (1416 copulations, $\chi^2 = 37.99$, $P < 10^{-5}$). Note however that most between-region pairwise tests showed positive assortative mating, such that there was no significant heterogeneity among different pairwise tests ($G_{Sex.Isol.Heterog.(between)} = 18.31$; df $= 34$; $P = 0.985$).

When we analyzed all 18 United States Bahamas mating tests further, we found that on average, homotypic United States and Bahamas pairs comprised 25% and 29% of all matings, respectively, whereas the reciprocal heterotypic pairs made up 23% and 22% of all matings (Fig. 2B). This result indicates that the strength of sexual isolation is asymmetrical with the Bahamas individuals showing stronger mating preferences compared to United States individuals (Estimation of Asymmetry "I_{apsi}" = 1.0119; SD 0.0044; $P = 0.0016$; Rolán-Alvarez and Caballero 2000). Similar asymmetry is observed for the significant pairwise United States–Bahamas tests (average: 27%$_{US}$ and 31%$_{Bahamas}$ homotypic matings, 21% heterotypic matings).

We further investigated whether the observed sexual isolation between United States and Caribbean populations could be of the same type as that of the Zimbabwe case or if this represents a distinct case of sexual isolation. In addition to comparing our results to Zimbabwe isofemale lines, we also tested our populations against West African (Cameroon) isofemale lines. It has been hypothesized that West African populations may have been the primary colonization source of Caribbean populations (e.g., David and Capy 1988; Caracristi and Schlotterer 2003).

Table 1. Pairwise multiple-choice mating tests of sexual isolation within and between United States and Bahamas regions. Numbers of copulations for each type are shown from left to right locations as (n_{aa}, n_{ab}, n_{ba}, n_{bb}), with the left subscript designating females and the right subscript males. Abbreviations are for number of copulations, number of replicates, and percent homotypic matings.

Id nos.	No. of copul.	No. of repl.	(N_{aa}, n_{ab}, n_{ba}, n_{bb})	% homot.	I_{psi} index	SD	GI or χ^2 values	P-value
Within United States								
15–19	190	6	(57, 50, 43, 40)	0.511	0.021	0.073	0.040	0.841
13–17	104	4	(25, 21, 30, 28)	0.510	0.019	0.098	0.071	0.8
21–22	68	2	(10, 22, 17, 19)	0.426	−0.147	0.120	1.820	0.151
22–23	169	5	(42, 41, 40, 46)	0.521	0.041	0.077	0.283	0.582
13–22	186	5	(60, 45, 41, 40)	0.538	0.075	0.073	0.785	0.363
Within Bahamas								
36–38	128	4	(31, 29, 36, 32)	0.492	−0.016	0.088	0.021	0.962
30–32	67	2	(20, 20, 13, 14)	0.507	0.015	0.122	0.022	0.878
29–39	185	6	(57, 45, 46, 37)	0.508	0.016	0.074	0.004	0.926
35–41	227	7	(53, 54, 58, 62)	0.507	0.013	0.066	0.033	0.844
33–37	127	4	(44, 39, 26, 18)	0.488	−0.024	0.089	0.430	0.522
33–45	178	5	(36, 43, 47, 52)	0.494	−0.011	0.075	0.064	0.782
Between United States and Bahamas								
19–34	246	7	(77, 48, 58, 63)	0.569	0.138	0.063	4.650	**0.021**
13–36	244	8	(69, 44, 60, 71)	0.574	0.154	0.063	5.700	**0.013**
22–33	176	6	(34, 47, 32, 63)	0.551	0.088	0.075	1.282	0.268
20–40	181	6	(58, 38, 35, 50)	0.597	0.193	0.073	6.720	**0.009**
15–37	238	7	(47, 49, 59, 83)	0.546	0.075	0.065	1.273	0.275
21–32	288	9	(81, 60, 77, 70)	0.524	0.052	0.059	0.746	0.3876
22–35	172	6	(44, 33, 37, 58)	0.593	0.184	0.075	5.680	**0.016**
17–38	152	6	(41, 30, 42, 39)	0.526	0.059	0.081	0.530	0.4632
22–30	320	10	(74, 71, 71, 104)	0.556	0.106	0.056	3.503	**0.056**
16–45	175	5	(43, 37, 34, 61)	0.594	0.182	0.074	5.686	**0.014**
21–42	127	4	(32, 36, 27, 32)	0.504	0.013	0.089	0.021	0.885
20–37	129	5	(30, 37, 29, 33)	0.488	−0.020	0.088	0.052	0.8174
18–44	224	7	(50, 44, 63, 67)	0.522	0.047	0.067	0.488	0.4906
23–36	328	9	(80, 87, 73, 88)	0.512	0.027	0.055	0.216	0.623
23–41	177	5	(51, 42, 32, 52)	0.582	0.169	0.074	5.000	**0.02**
22–45	180	6	(48, 33, 40, 59)	0.594	0.190	0.074	6.370	**0.011**
23–44	256	8	(57, 65, 66, 68)	0.488	−0.023	0.062	0.164	0.661
21–43	131	4	(36, 28, 39, 28)	0.489	−0.020	0.087	0.051	0.822

Notes: **Bold values designate significant (P < 0.05) or marginally significant P-values. All P-values were determined using JMATING software by bootstrapping 10,000 times (see text).**

RELATIONSHIP TO AFRICA

Table 2 gives the results of pairwise multiple-choice mating tests between North American and African populations. As expected, we found that United States populations were significantly sexually isolated from Zimbabwe (particularly from the strongly sexually divergent Sengwa population that was used by Wu et al. 1995; see Table 2). This result confirms previous reports of Wu et al. (1995) and Hollocher et al. (1997a) using our "populational" multiple-choice mating approach and is strong evidence that this approach is able to capture assortative mating in *D. melanogaster* in general (see *Materials and Methods*). The I_{psi} indexes between United States and Zimbabwe (Sengwa) were 0.189 and 0.292,

with an average I_{psi} index of 0.24 (SD = 0.05), which is significantly higher than the average I_{psi} index for all 18 United States-Bahamas pairwise tests (0.089; st. dev. 0.0017; Kruskal–Wallis test: $P = 0.038$), and higher than, but not significantly different from, the average I_{psi} index between the eight significant/marginally significant United States–Bahamas pairwise tests (0.164; st. dev. 0.02; Kruskal–Wallis test: $P = 0.09$). This suggests that United States-Bahamas sexual isolation is weaker than the isolation between the United States and Zimbabwe. However, the Zimbabwe population from Lake Kariba (Zim-k) did not show significant sexual isolation with United States and Bahamas flies (Table 2). This supports previous findings that mating preferences
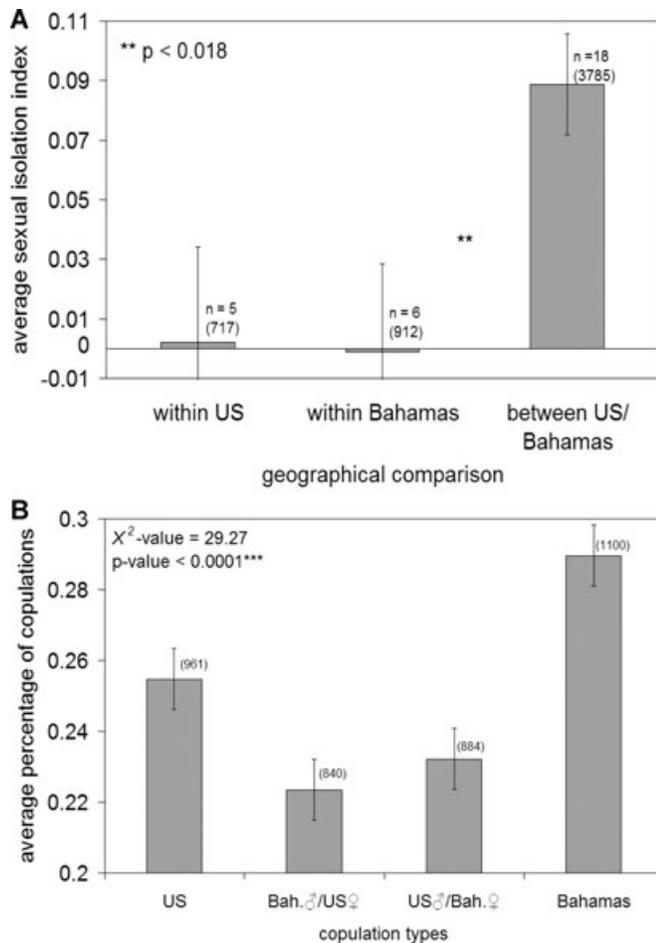
Figure 2. Average sexual isolation I_{psi} index for multiple-choice mating tests within United States, within Bahamas, and between United States and Bahamas localities (A). Average percent of copulations of homotypic and heterotypic pairs for between United States–Bahamas comparisons (B). The I_{psi} index in (A) was determined using the software JMATING (range: −1 to +1 with 0 = random mating; see text). For each geographic comparison, n represents the number of pairwise mating tests (see Table 1) and values in parentheses represent the total number of copulations accumulated. Error bars represent standard deviations.

in Zimbabwe are not fixed within populations (Hollocher et al. 1997a).

Mating tests between Bahamas islands and the Zimbabwe (Sengwa) population resulted in the average I_{psi} index of 0.159 (st. dev. 0.05) and only one of the tests was significant (Table 2). This suggests that Zimbabwe–Caribbean populations have weaker mating discrimination compared to United States–Zimbabwe populations. Using our Caribbean lines and the standard cosmopolitan French line FrV3–1, S. Fang (pers. comm.) also found that Caribbean females mate more readily with Zimbabwe males than with the French males. Despite relatively low sexual isolation between Bahamas and Zimbabwe lines, it is evident that these populations do not mate at random. Thus it is unlikely that sexual

isolation between United States-Caribbean populations is of the same type as between United States-Zimbabwe populations.

We performed mating tests between North American populations and a West African population from Cameroon. Interestingly, we found that United States–West African populations were significantly sexually isolated from one another with I_{psi} indexes of 0.202 and 0.17 for different replicates and an average I_{psi} index of 0.186 (Table 2). Even though this United States-West African case of sexual isolation is novel, these results are consistent with previous reports showing that some West African lines show sexual discrimination against other cosmopolitan lines (Cohet and David 1980; Scott 1994; Capy et al. 2000; Haerty et al. 2005; see Discussion). On the other hand, when we performed mating tests between Bahamas and West African populations, we found complete random mating (Table 2). This suggests that Caribbean and West African populations have similar mating preferences and/or mating cues.

If Caribbean and West African populations share mating preferences, we would expect significant sexual isolation between West Africa and Zimbabwe because partial sexual isolation was observed between Bahamas and Zimbabwe (see above). Interestingly, we found that the sexual isolation between West African and Zimbabwe (Sengwa) populations was the highest observed in our study with I_{psi} index of 0.282 (Table 2). This suggests that West African and Zimbabwe lines have diverged substantially in their mating preferences. We summarize our mating choice results in Figure 3, which shows the average isolation indexes of relevant regional tests.

Discussion
INCIPIENT SEXUAL ISOLATION BETWEEN COSMOPOLITAN POPULATIONS

Our results provide evidence of incipient sexual isolation occurring between cosmopolitan strains of *D. melanogaster*. Because cosmopolitan populations are generally assumed to not deviate from random mating, we discuss some of the potential reasons for our observations. We note that the original paper studying multiple-choice mating tests of worldwide *D. melanogaster* populations did not sample any lines from the Caribbean or West Africa (Henderson and Lambert 1982). Subsequent work by Wu et al. (1995) and Hollocher et al. (1997a) only looked at a single West African line (from Tai, Ivory Coast), which showed a significant level of sexual isolation from Zimbabwe lines and was therefore taken as a representative of cosmopolitan mating behavior. Even though our results are consistent with these observations, they also indicate that United States and West African populations are partially sexually isolated.

Interestingly, the original paper that described divergent mating behavior between African and cosmopolitan flies discovered

Table 2. Pairwise multiple-choice mating tests of sexual isolation between United States and Bahamas and Zimbabwe and West African populations.

| Id nos.	No. of copul.	No. of repl.	(N_{aa}, n_{ab}, n_{ba}, n_{bb})	% homot.	I_{psi} index	SD	GI or χ^2 values	P-value
Between Zimbabwe								
and United States								
21-Zim(s)	217	7	(78, 53, 35, 51)	0.594	0.189	0.067	7.420	**0.003**
19-Zim(s)	154	5	(65, 33, 21, 35)	0.649	0.292	0.081	12.009	**0.0006**
16-Zim(k)	184	6	(67, 31, 48, 38)	0.571	0.136	0.076	3.080	0.076
and Bahamas								
45-Zim(s)	202	7	(79, 43, 38, 42)	0.599	0.177	0.072	5.902	**0.016**
35-Zim(s)	150	5	(51, 35, 29, 35)	0.573	0.141	0.082	2.885	0.0864
41-Zim(k)	193	7	(56, 37, 62, 38)	0.487	−0.018	0.072	0.065	0.8122
Between West								
Africa (Cameroon) and United States								
22-Cam	198	6	(67, 36, 43, 52)	0.601	0.202	0.070	7.835	**0.004**
19-Cam	205	6	(59, 35, 51, 60)	0.580	0.170	0.070	5.791	**0.011**
and Bahamas								
45-Cam	179	6	(57, 43, 47, 32)	0.497	−0.026	0.077	0.113	0.747
35-Cam	192	6	(57, 44, 53, 38)	0.495	−0.019	0.075	0.064	0.804
Between Zimbabwe								
and West Africa (Cameroon)								
Cam-Zim(s)	156	6	(56, 39, 17, 44)	0.641	0.282	0.077	14.411	**0.0009**

Note: See description in Table 1.

the phenomenon in an isofemale line from a West African population (Brazzaville, Congo) that is geographically adjacent to Cameroon (Cohet and David 1980; see also Capy et al. 2000; Haerty et al. 2005). Because the Congo line has not been directly tested against Zimbabwe lines, it is not clear whether it is also sexually isolated from Zimbabwe. Similar evidence comes from the West African Tai, Ivory Coast isofemale line, which was shown to be partially sexually isolated from the cosmopolitan Canton-S isofemale line (Scott 1994; Cobb and Ferveur 1996; Grillet et al. 2006; but see Coyne et al. 1999). This may mean that both the Congo and the Ivory Coast mating preferences may ultimately be of the same type as the Cameroon case described here. The partial sexual isolation between West African and Zimbabwe is consistent with evidence indicating that West and East (including Zimbabwe) African strains make up two genetically distinct groups (e.g., Bénassi and Veuille 1995; Aulard et al. 2002; Baudry et al. 2004; Pool and Aquadro 2006).

The only previous study to examine mating preferences of Caribbean populations was by Fang et al. (2002). These authors analyzed four isofemale lines (one from Cuba, one from Dominican Republic, and two from St. Croix). These results showed that these Caribbean lines were more M-like (cosmopolitan) than Z-like in mating preferences, resembling lines from Central and West Africa (see Hollocher et al. 1997a). However, partial sexual isolation was described in a recent study of mating behavior of *desaturase*-2 pheromone locus transgenic lines (lines that

carry the Zimbabwe insertion allele in an otherwise cosmopolitan background; Coyne and Elwyn 2006). These authors argued that the transgenic lines may represent Caribbean-like genotypes because some Caribbean populations also carry the insertion allele at this pheromone locus, but are otherwise presumed to have a cosmopolitan genetic background (Takahashi et al. 2001; Fang et al. 2002; Coyne and Elwyn 2006). Our results of partial sexual isolation between United States and Caribbean populations are consistent with these findings. However because we observe geographical variation for incipient sexual isolation in this system, it is not surprising that Fang et al. (2002) found weak sexual isolation in their lines. Strongly sexually isolated isofemale lines in the Caribbean, if these exist at all, would have been difficult to find without prior geographical knowledge of variation in mating preferences.

HOW AFRICAN ARE CARIBBEAN FLIES?

Clearly our results raise new questions about the genetic and phenotypic makeup of Caribbean populations and their relationship to African strains. The finding that Caribbean and West African populations mate randomly with each other, but are sexually isolated from both United States cosmopolitan and Zimbabwe strains suggests that this case of sexual isolation is likely to have a distinct genetic and phenotypic basis from that of Zimbabwe. This may mean that both Caribbean and West African flies prefer certain phenotypes that are absent in cosmopolitan and Zimbabwe strains
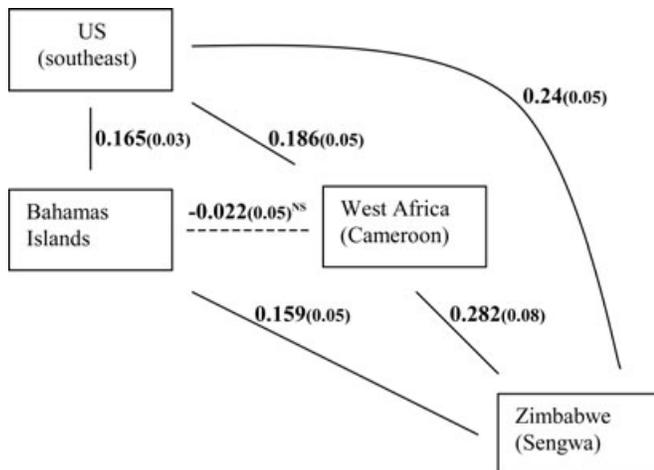
Figure 3. Average pairwise sexual isolation (I_{psi}) indices from multiple-choice mating tests between populations of United States, Bahamas, West Africa, and Zimbabwe in Tables 1 and 2. Standard deviation of the sexual isolation index is shown in parentheses (all indices except Bahamas–Cameroon, are significant at $P < 0.0001$). The averages are based on the following number of pairwise comparisons (and number of copulations): United States–Bahamas: 7(1416), United States–Zimbabwe: 2(371), United States–Cameroon: 2(403), Bahamas–Zimbabwe: 2(352), Bahamas–Cameroon: 2(371), Cameroon–Zimbabwe: 1(156). Note the average I_{psi} index of 0.164 is only for the eight significant and marginally significant pairwise tests between United States and Bahamas (see text). If all 18 pairwise tests were used, the average I_{psi} index would be 0.089 (0.02) for United States–Bahamas. Similarly, only pairwise tests with Zimbabwe (Sengwa) were used, not Zimbabwe (Kariba) because our interest is to compare our results to the strong sexual isolation of Zimbabwe (Sengwa) described by Wu et al. (1995) and Hollocher et al. (1997a). If Zimbabwe(k) is also included, the average I_{psi} index would be 0.206 (0.04) for United States–Zimbabwe, and average I_{psi} index = 0.099 (0.04) for Bahamas–Zimbabwe. Cameroon–Zimbabwe(k) isolation was not tested.

or that these flies prefer intermediate trait values between extreme cosmopolitan and Zimbabwe phenotypes.

The observation that some Caribbean populations harbor exceptional African-like phenotypes and pheromones has led some to suggest that Caribbean flies may have had direct origins from Africa via historical trans-Atlantic slave trades (David and Capy 1988; Capy et al. 1993, 1994; Rouault et al. 2001; Takahashi et al. 2001; Caracristi and Schlotterer 2003; Kauer and Schlotterer 2004, but see Capy et al. 1986). Our sexual isolation patterns are consistent with this historical scenario, but it is certainly possible that Caribbean populations may represent convergent evolution onto African-like tropical phenotypes and mating preferences. We have begun to characterize in detail phenotypic and behavioral variation associated with incipient sexual isolation between United States, Caribbean, and African populations to determine whether these cases of incipient sexual isolation are associated

with similar or different trait divergence (Yukilevich and True, in press). Future work should also focus on phylogenetic relationships among the United States, Caribbean, and African populations using genetic markers and sequences (see also Caracristi and Schlotterer 2003). Finding a genetic signature of African (especially West African) ancestry in the Caribbean would clearly support the hypothesis that Caribbean populations are recent, direct descendents of African populations and that the mating preferences in the Caribbean are likely to be the same as those in West Africa.

IMPLICATIONS FOR SEXUAL ISOLATION IN WIDESPREAD AND HUMAN-COMMENSAL SPECIES

These results extend our understanding of the evolution of incipient sexual isolation within recently expanding widespread and human-commensal species. Previous findings of incipient sexual isolation between Zimbabwe and cosmopolitan populations indicated that species with expanding geographical ranges and those associated with humans are capable of rapid divergence in sexual mating preferences and phenotypic cues (Wu et al. 1995; Hollocher et al. 1997a). However, it was generally assumed that populations from the rest of the species range mate randomly and do not segregate mating preference behaviors.

Our results indicate otherwise, by showing that populations in Caribbean and West Africa exhibit incipient sexual isolation with other cosmopolitan populations in United States and that these mating preferences are likely to be distinct from those of Zimbabwe. This suggests that more than one type of mating preference has evolved in *D. melanogaster*. These results contribute to a rapidly changing view of the evolutionary history and biology of *D. melanogaster*, which was originally thought to be a genetically undifferentiated and panmictic unit (see David and Capy 1988 for details). These findings imply that widespread and human-commensal species can segregate genetic and phenotypic variation for mating preferences and initiate the process of sexual isolation in different parts of the species range.

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