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I am submitting herewith a thesis written by Jessica Marie Bier entitled "Mating Behavior of *Drosophila affinis*: a Test for Geographic Variation in Intraspecific and Interspecific Mate Discrimination." I have examined the final copy of this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Ecology and Evolutionary Biology.

Christine R. B. Boake
Christine R.B. Boake, Major Professor

We have read this thesis and
recommend its acceptance:

Michael P. Cyp
Donald M. Byrnes
Jonathan C. Edwards

Accepted for the Council:

Levon Minkal
Associate Vice Chancellor and
Dean of The Graduate School

Mating Behavior of *Drosophila affinis*: a Test
for Geographic Variation in Intraspecific
and Interspecific Mate Discrimination

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ABSTRACT

Two general models of the evolution of courtship behavior have been proposed; one emphasizes evolutionary stability while the other emphasizes evolutionary lability of mating signals and preferences. The documentation of geographic variation in mating behavior in a wide range of taxa provides support for the view that preferences and signals are subject to rapid evolution. A lack of geographic variation in mating behavior has also been documented for several species which suggests that the validity of the two opposing models of the evolution of courtship may depend on the system in question. Additional case studies are required to determine the generality of interpopulation divergence in mating behavior and to allow for comparisons of patterns between species.

In this study, I tested for the presence of geographic variation in *Drosophila affinis*, a woodland North American species. A total of 63 multiple mate choice tests were run between 21 *D. affinis* isofemale lines from eight geographic populations and a *D. affinis* tester stock from Tennessee. *Drosophila affinis* females have previously been reported to mate with a closely related species, *D. athabasca*, in the laboratory, suggesting the possibility of influence on mating behavior from interspecific interactions. To test for the presence of geographic variation in interspecific discrimination, I ran a series of multiple choice tests between nine *D. affinis* isofemale lines from seven geographic populations and a tester stock of *D. athabasca* from Tennessee.

There was no significant variation in *D. affinis* in either intraspecific and interspecific discrimination, which is noteworthy for two reasons. First, this study is one of the few that reports a lack of such variation in a *Drosophila* species. Second, extensive geographic variation in mating behavior has previously been documented in *D.*

athabasca, an ecologically similar, close relative of *D. affinis*. I discuss possible reasons for the different patterns of variation found in the two species.

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I. INTRODUCTION

Mating Behavior and Speciation

Courtship behavior is one of the most important defining characters of a species. First, it acts as a cohesive force by leading to reproduction between individuals with a coordinated set of signals and responses. Second, it serves as a barrier to reproduction between individuals with variant sets of signals and responses. In other words, the *function* of mating behavior is to coordinate reproduction between conspecifics while the *effect* of species-specific mating behavior is a barrier to reproduction between heterospecifics (Ryan 1990). Although there are several species concepts that emphasize different processes and patterns (reviewed by Templeton 1989), reproductive isolation is the most widely accepted criterion used to define species boundaries. Because differences in courtship behavior contribute to premating isolation, an understanding of the evolution of courtship behavior is critical to our understanding of its relative contribution to the process of speciation.

The traditional model of the origin of reproductive isolation viewed behavioral isolation, which is attributable to divergent mating signals and preferences, as a result of divergence in other characters (Mayr 1963). Courtship behavior diverged either as an incidental by-product of genetic divergence or in response to selection against unfit hybrids in zones of sympatry. Under this model, mating signal divergence was assigned a secondary role in the origin of reproductive isolation. More recently, it has been recognized that premating isolation due to differences in courtship behavior may play a more fundamental role in the speciation process. There is a growing amount of evidence that differences in courtship behavior can serve as the basis of mating incompatibility

between populations that have diverged very little in nonreproductive characters (West-Eberhard 1983; Coyne & Orr 1989). The relative importance of mating behavior in the origin and maintenance of diversity is in part dependent on the rate of evolution of signals and preferences relative to other factors that may lead to isolation.

The Evolution of Mating Signals and Preferences

Two general verbal models describe the evolution of mating signals and preferences (Butlin 1995a). The first model emphasizes stability of mating signals both within and between conspecific populations and assumes that selection on signals and preferences is primarily stabilizing. This view is exemplified by Paterson's (1985) concept of courtship, the Specific Mate Recognition System (SMRS). The SMRS requires mutual recognition and therefore the most efficient signal (and response) will be the one that corresponds to the mean of the population. Because individuals with variant signals and preferences will fail to mate, intense stabilizing selection is predicted (Paterson 1985). This model is concordant with the historical typological concept of species that did not acknowledge variation within species (Verrell 1999). In addition, the traditional view of courtship as a mechanism to prevent interspecific matings predicted selection for invariant signals (Verrell 1999).

The second model emphasizes the mutability of mating signals, primarily as a consequence of intersexual selection. Several properties of sexual selection lead to the prediction of evolutionary lability and rapid interpopulation divergence of signals and preferences (West-Eberhard 1983). First, sexually selected characters are likely to be subject to strong selection in every generation. Second, there is a potential for extreme exaggeration of characters. In addition, there is a potential for a runaway process in

which a genetic correlation between a preference and a trait leads to rapid evolutionary change in both (Fisher 1958; for a complete discussion of special properties of sexual selection see West-Eberhard 1983). Intersexual selection has been documented in a wide range of species (reviewed by Andersson 1994). Therefore, if sexual selection has the potential to accelerate divergence in signals and preferences, we may expect that such divergence plays a key role in speciation processes. Models relating sexual selection and speciation show that variation in female preferences can result in the rapid evolution of both trait and preference, resulting in reproductive isolation (e.g. Lande 1981; Turner & Burrows 1995; Payne & Krakauer 1997).

Other forces besides sexual selection may lead to divergence in courtship behavior (and therefore reproductive isolation) between populations. Interpopulation differences in signals and preferences have been found to result from the following: occupation of habitats with different signal transmission properties, exposure to different levels of predation pressure, and as a correlated response to selection on other characters (reviewed by Verrell 1999). In addition, reproductive interactions between closely related taxa that have diverged in adaptive characters may lead to selection against heterospecific mating in sympatric populations, a process known as "reinforcement" when hybrids are produced or "reproductive character displacement" when hybrids are not produced (Butlin 1989, but see Howard 1993). The importance of reinforcement in the enhancement of reproductive isolation has been intensely debated but data exist that support the plausibility of its occurrence (Coyne and Orr 1989; Noor 1995; Littlejohn 1999).

Although several factors may lead to changes in signals and preferences between populations, sexual selection is the one force that does not require differences in the biotic or physical environment. For this reason and because of its special properties (see above) sexual selection may play a relatively important role in the inception of reproductive isolation.

Geographic Variation in Mating Behavior

One prediction generated by the idea that sexual selection can lead to rapid evolution of preferences and traits is the extensive occurrence of geographic variation in courtship behavior across the range of a species in the absence of any ecological variation that would lead to selection for different signals (Butlin 1995a). The stabilizing view of signal evolution makes the opposite prediction. Paterson (1982) proposed that change in environmental conditions is the only factor that could promote changes in signals and responses, so unless different environments are encountered, SMRS characters are expected to remain stable across the range of a species.

The study of geographic variation in mating behavior should help elucidate which model of mating signal and preference evolution is most realistic. Butlin (1995a) reviewed descriptions of variation in mating characters between conspecific populations. Excluding studies of hybrid zones, out of 87 studies, a lack of variation was reported in only 18 cases. Less than half of the studies that reported significant geographic variation tested for the effect of this variation on mate choice so it is difficult to assess the biological significance of the variation in relation to reproductive isolation.

The genus *Drosophila* has been the genus of choice for studies of geographic variation, comprising a third of the studies reviewed by Butlin (1995a). Within this

genus, 29 of 34 tested species showed significant geographic variation in mating signals and preferences which was indicated by the presence of assortative mating in a majority of the studies (Butlin 1995a).

The data available suggest that geographic variation may be the rule rather than the exception. One problem with this conclusion is that the absence of variation is less likely to be reported (Butlin 1995a). It is evident that variation in mating behavior leading to isolation is present in many taxa that appear to lack variation in other characters (Wells & Henry 1998). This supports the validity of the view that the evolution of signals and preferences can be relatively rapid and therefore a potentially critical component of speciation processes.

Although the debate surrounding the two models of signal evolution discussed above has generally treated them as mutually exclusive, their validity may be dependent on the system in question. The concept of "courtship" covers a huge range of behaviors that vary widely in modality and complexity. Genetic control which may influence the rate of evolution (Verrell 1999) is sure to vary across taxa, although relatively few studies have examined the genetic basis of behavioral isolation (Coyne & Orr 1998; Hollocher 1998). Due to the diverse and multifaceted nature of courtship behavior, we might expect its rate of evolution to be diverse across taxa as well. A more complete survey of geographic variation in courtship behavior may allow us to identify the critical factors that determine the relative rate of signal and preference evolution. For example, it may be that certain signal modalities (and corresponding preferences) are more susceptible to change.

Knowledge of the extent and generality of interpopulation divergence in sexual characters is needed to help determine the relative importance of behavioral isolation in the generation of diversity. With this need in mind, I conducted a study of geographic variation in *Drosophila affinis*, a North American woodland species, by testing for assortative mating between several geographic populations. The choice of *D. affinis* was in part based on availability and ease of collection. In addition, extensive geographic variation has been documented in another member of the *affinis* subgroup, *D. athabasca*. *Drosophila affinis* and *D. athabasca* are closely related with low levels of genetic divergence relative to other estimates between *Drosophila* species (0.96-1.30 % mtDNA sequence divergence (Yoon & Aquadro 1994)), occupy similar habitat, and overlap in distribution in the eastern United States (Miller 1958; Hey & Houle 1987). I was primarily interested in intraspecific discrimination but also conducted a series of mate choice tests between *D. affinis* and *D. athabasca* to test for possible effects of interspecific interactions. Documentation of either the presence or absence of geographic variation in *D. affinis* is valuable as a case study. It also provides an opportunity to make a comparison between two closely related, ecologically similar species.

Description of Species and Review of Previous Studies

Drosophila affinis and *D. athabasca* are members of the *affinis* subgroup (*Obscura* group) which includes eight North American species. Species of the *affinis* subgroup are the most abundant native *Drosophila* in eastern North America (Miller 1958). *Drosophila affinis* is generally limited to the eastern half of the United States, extending north into Minnesota and south into Florida. *Drosophila athabasca* is not found in the most southern states but has a range that extends farther north (into Canada)

and west (to the Pacific Coast) than the range of *D. affinis* (Miller 1958). Collecting has not been intensive nor systematic so it is not known whether the distributions are continuous or where the exact limits of the ranges occur. In addition, the morphological similarity between *D. affinis* and *D. athabasca* has probably led to misidentification (Miller 1958).

Relatively little is known about the ecology of the two species. They are regularly found in both coniferous and deciduous forests and are considered to be woodland species (Miller 1958; Miller and Weeks 1964; Hey and Houle 1987; Yoon 1991). Low numbers of larvae of both species have been raised out of fleshy fungi, small fruits, and tree bleeds (Miller 1958; Yoon 1991). The distribution and relative frequencies of *D. affinis* and *D. athabasca* in collections has been linked to temperature and humidity effects. Laboratory studies have shown that *D. affinis* is able to withstand higher temperatures and lower levels of humidity than *D. athabasca* (Richmond 1980; Fogleman 1982), which helps to explain the change in relative frequencies of *D. affinis* group species correlated with change in latitude, elevation, and season (Miller 1958). *Drosophila affinis* decreases in frequency with increasing latitude while *D. athabasca* shows the inverse pattern (Miller 1958). The relative frequency of *D. affinis* is at the maximum in the middle of the summer and numbers of *D. affinis* and *D. athabasca* decrease and increase respectively with increasing elevation in the Great Smoky Mountains (Miller 1958).

Members of the *affinis* group have been used to test several evolutionary and ecological hypotheses (Gromko et al. 1980; Hey and Houle 1987; Ford and Aquadro 1996) and mating behavior of both *D. affinis* and *D. athabasca* has been relatively well

studied (Miller 1950a, 1950b; Chang & Miller 1978; McRobert & Tompkins 1986; Yoon 1991). Courtship behaviors found in the two species are typical of many *Drosophila* (Miller 1950b; McRobert & Tompkins 1986; personal observation). The male orients towards the female, vibrates one or both of his wings, and may circle from the front to the rear of the female several times. The female may respond with wing vibration or spread her wings to indicate a willingness to mate. Individuals may touch each other with their front legs although this does not appear to be a necessary component of courtship. Wing vibration produces low and high pulse repetition sounds which are described as a “fluttering staccato” and “buzzing burst” in *D. athabasca* whose sounds are louder than those produced by *D. affinis* (Chang & Miller 1978). Geographic variation in pulse repetition rate has been found in *D. athabasca* and is thought to be a factor contributing to high levels of premating isolation between populations (Chang & Miller 1978; Yoon 1991).

The levels of reproductive isolation between populations of the *D. athabasca* complex prompted the division of the complex into 3 semispecies, Western-Northern (WN), Eastern-A (EA), and Eastern-B (EB) (Miller & Westphal 1967; Johnson 1985; Yoon & Aquadro 1994). Isolation between WN and EA and between EA and EB is high (fewer than 10% successful matings) and not as complete between WN and EB (fewer than 40% successful matings) (Yoon & Aquadro 1994). The amount of genetic divergence between the semispecies is very low (Johnson 1985; Yoon & Aquadro 1994) and the high levels of isolation are thought to have arisen in the last 20,000 years (Ford & Aquadro 1996). Although the exact distribution of the semispecies remains in question, the deduced pattern of range overlap, the level of isolation between the three semispecies,

and results from genetic studies led Ford and Aquadro (1996) to propose that reinforcement has played a role in this system. Reinforcement is a highly debated issue (Butlin 1995b) but some of the most convincing evidence for the plausibility of the process has come from studies of *Drosophila* (Coyne & Orr 1989; Noor 1995).

Research Overview

The geographic variation in mating signals and preferences that is present in *D. athabasca* suggests that such variation may also be present in *D. affinis*, which is ecologically similar and a close relative. To test for the presence of geographic variation in mating behavior in *D. affinis*, I ran a series of mate choice tests using multiple isofemale lines from eight geographic locations. These lines were started from individual females collected during the summer of 1998. I used a multiple mate choice design and each line was tested with a *D. affinis* “tester” line from Tennessee.

Drosophila affinis females have been reported to mate in the laboratory with *D. athabasca* males with a small number of hybrids produced (Miller 1950a). This suggests the possibility that reinforcement may play a role in the evolution of signals and preferences in this group due to selection against heterospecific matings. To test whether different populations of *D. affinis* had varying levels of discrimination against *D. athabasca*, I ran a series of multiple choice tests using a subset of the lines used in the intraspecific tests. The *D. affinis* lines were all tested with a *D. athabasca* “tester” line from Tennessee. Variation in levels of discrimination may be expected in *D. affinis* populations depending on the rate of encounter with *D. athabasca* individuals. In addition, different sympatric populations are likely to encounter *D. athabasca* males with

different mating signals. These tests were exploratory in nature and not designed to provide conclusive evidence for the process of reinforcement.

II. METHODS

Collecting

I collected flies used to start isofemale lines from May to August 1998 from seven sites in six states in the eastern and midwestern United States (Figure 1; Table 1, Appendix). Most sites were densely forested with primarily deciduous trees, generally with well developed groundcover and understory. One exception was the South Dakota (SD) site where the trees were sparse and surrounded by grassland. Plastic containers (Figure 2) filled with rotting bananas were suspended from tree branches 1.2-1.8 m above the ground. The bottom of the plastic container had been replaced with a mesh that allowed the flies access to the bananas. I collected flies by aspirating individuals off the mesh one to five days after the baits had been placed.

The females I collected were placed individually in food vials and I froze the males for later identification. Individuals were keyed to species using the key published by Sulerud & Miller (1966). There is a lack of reported characters to distinguish between the *D. affinis* group females so I identified females by examining at least 3 of their male offspring. An additional *D. affinis* isofemale line from Rosemont, Pennsylvania, started in 1993, was provided by Scott McRobert (Saint Joseph's University, Philadelphia PA).

Rearing

I started isofemale lines using the first generation offspring (10-30 of each sex) from each female that had been collected in the field. For each generation, each line was maintained in 4-6 vials. To start a new generation, I transferred newly emerged individuals to fresh food vials (15-30 flies/vial). The individuals in each fresh food vial were generally taken from at least two of the old vials; thus isofemale lines were not

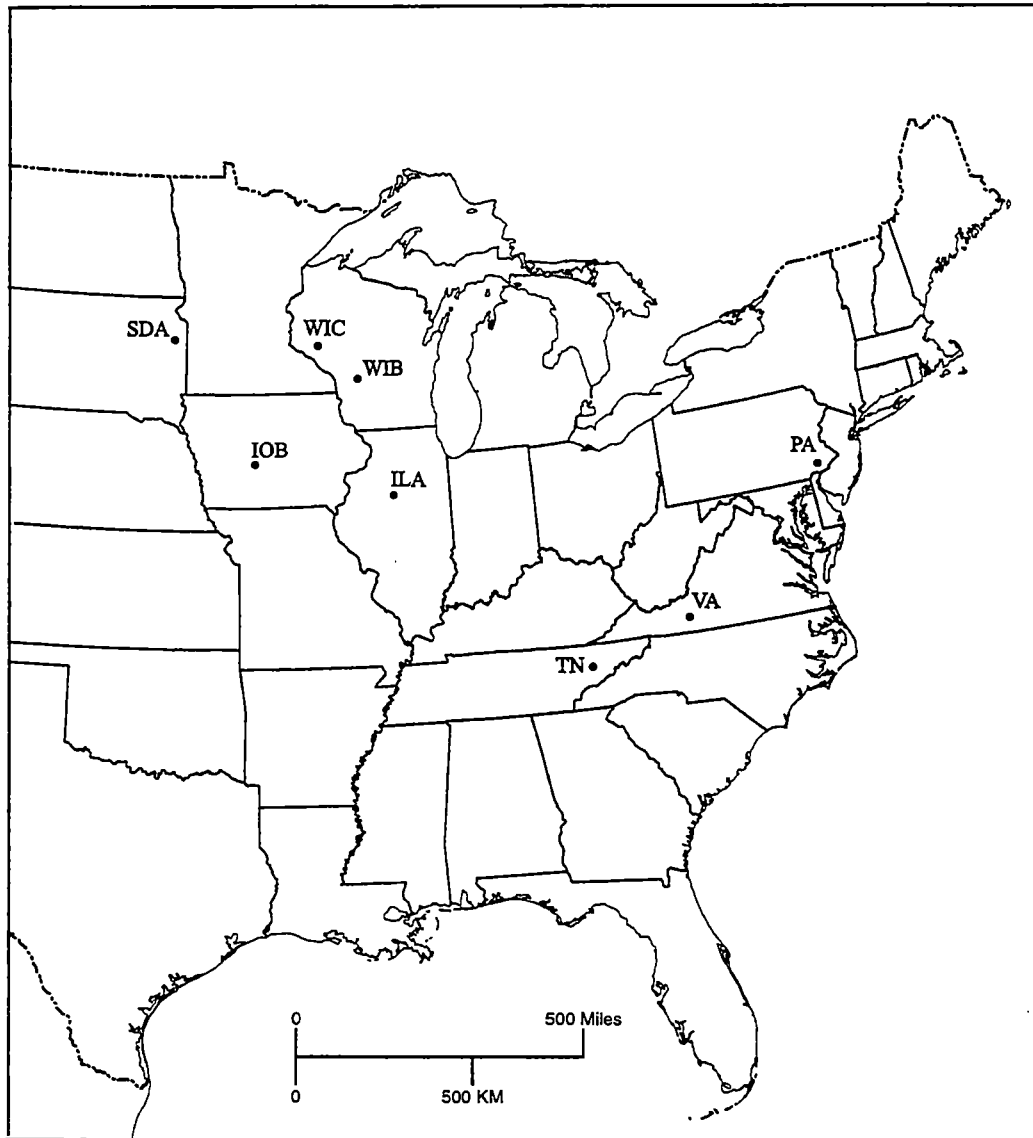


Figure 1. Location of sites and their code designations where *Drosophila affinis* and *Drosophila athabasca* females were collected.

Table 1. Lines used in multiple mate choice tests. All lines are *Drosophila affinis* except TN4 which is *D. athabasca*.

Site designation	Location	Lines used in intraspecific tests (xTN2)	Lines used in interspecific tests (xTN4)	Date collected
ILA	Peoria, Illinois	ILA2, ILA4, ILA7, ILA9	ILA2, ILA4	June 1998
IOB	Ledges State Park, Iowa	IOB2		June 1998
PA	Rosemont, Pennsylvania	PA	PA	1993*
SDA	Watertown, South Dakota	SDA1, SDA13, SDA18, SDA19, SDA21	SDA1, SDA13	June 1998
TN	Knoxville, Tennessee	TN5	TN2, TN4	August 1998
VAC	Ferrum, Virginia	VAC1, VAC5, VAC11, VAC12	VAC1, VAC5	May 1998
WIB	Black River State Forest, Wisconsin	WIB1, WIB4, WIB7		June 1998
WIC	Eau Galle Day Use Area, Wisconsin	WIC3, WIC42	WIC3, WIC42	June 1998

*provided by Scott McRobert (St. Joseph's University, PA)

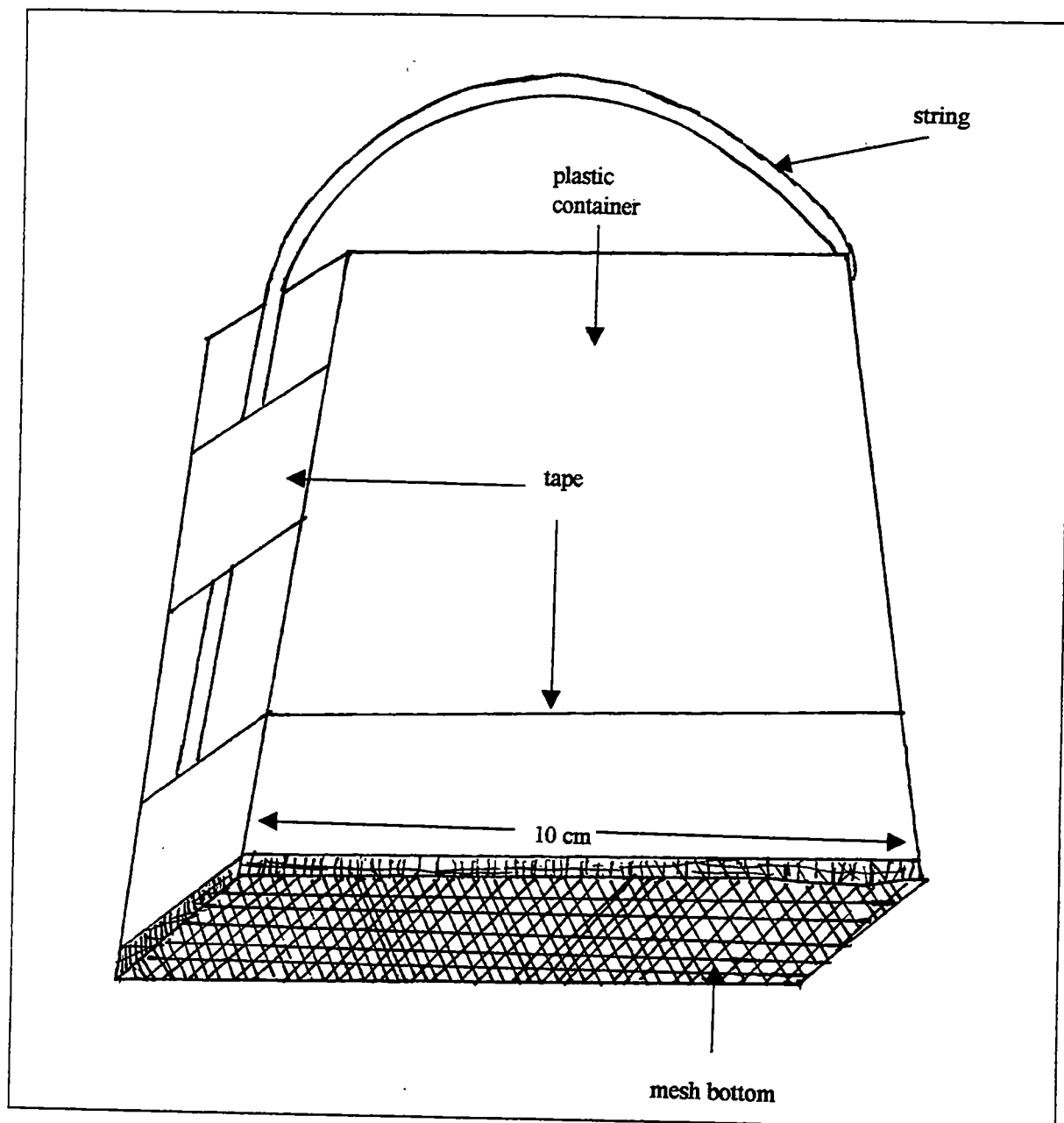


Figure 2. Bait container. A plastic container (1 L) was filled with two to three rotten bananas. The opening was covered with a fine mesh that excluded the flies from the interior of the container but allowed the flies access to the bananas. The container was suspended from a tree branch 1.2 to 1.8 m above the ground. Using an aspirator, I collected flies off the mesh one to three days after the bait had been placed.

subdivided. Mold growth often inhibited offspring production in some of the vials, but I attempted to start each generation with individuals collected from as many vials as possible.

Instant *Drosophila* food (Carolina Biological Supply Company) was used as a food medium and 0.05% propionic acid solution was used to dampen the medium in place of water to discourage fungal growth. Paper strips were placed in the vials to provide pupation sites. Flies were kept on a 12L:12D cycle at 18° C.

Tests of intraspecific mate discrimination

There are two alternative approaches to studying variation in mating signals and preferences. The first is to measure signal characters that are involved in courtship and test for differences at the level of the individual, population, or species. The second approach is to use the mating patterns resulting from choice tests as evidence for signal and preference divergence. Random mating in choice tests indicates a lack of evolutionarily significant differences between the tested groups whereas assortative mating is evidence of divergence in signals and/or preferences.

I used the second approach to test for geographic variation in mating signals and preferences in *D. affinis*. There are several types of mate choice tests; I chose to use multiple choice tests because my field observations indicated that this type of test may best represent natural conditions. Individual females present on the bait containers were often courted by several males at the same time. I conducted multiple mate choice tests using isofemale lines from the eight geographic populations described in Table 1, Appendix. In order to account for intrapopulation variation, I used multiple isofemale lines from most locations. All lines were tested with a "tester" line of *D. affinis* from

Knoxville, Tennessee (TN2) and each line combination was replicated three times. At least one of the three replicates contained individuals from a different generation and with one exception, no two replicates were run on the same day. The tests were conducted during January through April 1999. With the exception of the PA line, the lines used in the tests were maintained in the laboratory for 12-17 generations prior to testing. I estimate that the PA line has been in captivity for approximately 80 generations.

Virgin flies were collected within 24 hrs after emergence (Miller 1950b). Using CO₂ and a cold pack, I lightly anaesthetized flies to sort males and females (using a dissecting microscope) and transferred them to separate vials (15-30 flies/vial). They were aged 6-10 days before use in mate choice experiments. Twenty-four to 48 hrs before being tested, flies of each line were transferred to food vials containing 4-6 drops of either yellow or blue food coloring. In at least one of the three test replicates, the color assigned to each of the two lines was reversed. The food coloring provided a means to determine the population identity of copulating pairs collected during the tests. Using a dissecting microscope, it was possible to see the colored food in the abdomen of the females. Males had to be dissected to determine the color of the gut contents.

Fifty females and 50 males of both the "tested" line and the "tester" line (TN2) were aspirated into a mating chamber (40 cm x 20 cm x 20 cm; Figure 3). The temperature in the chamber ranged from 19 C to 25 C and a humidity level of 60-75% was maintained by covering the bottom of the chamber with a wet paper towel. The

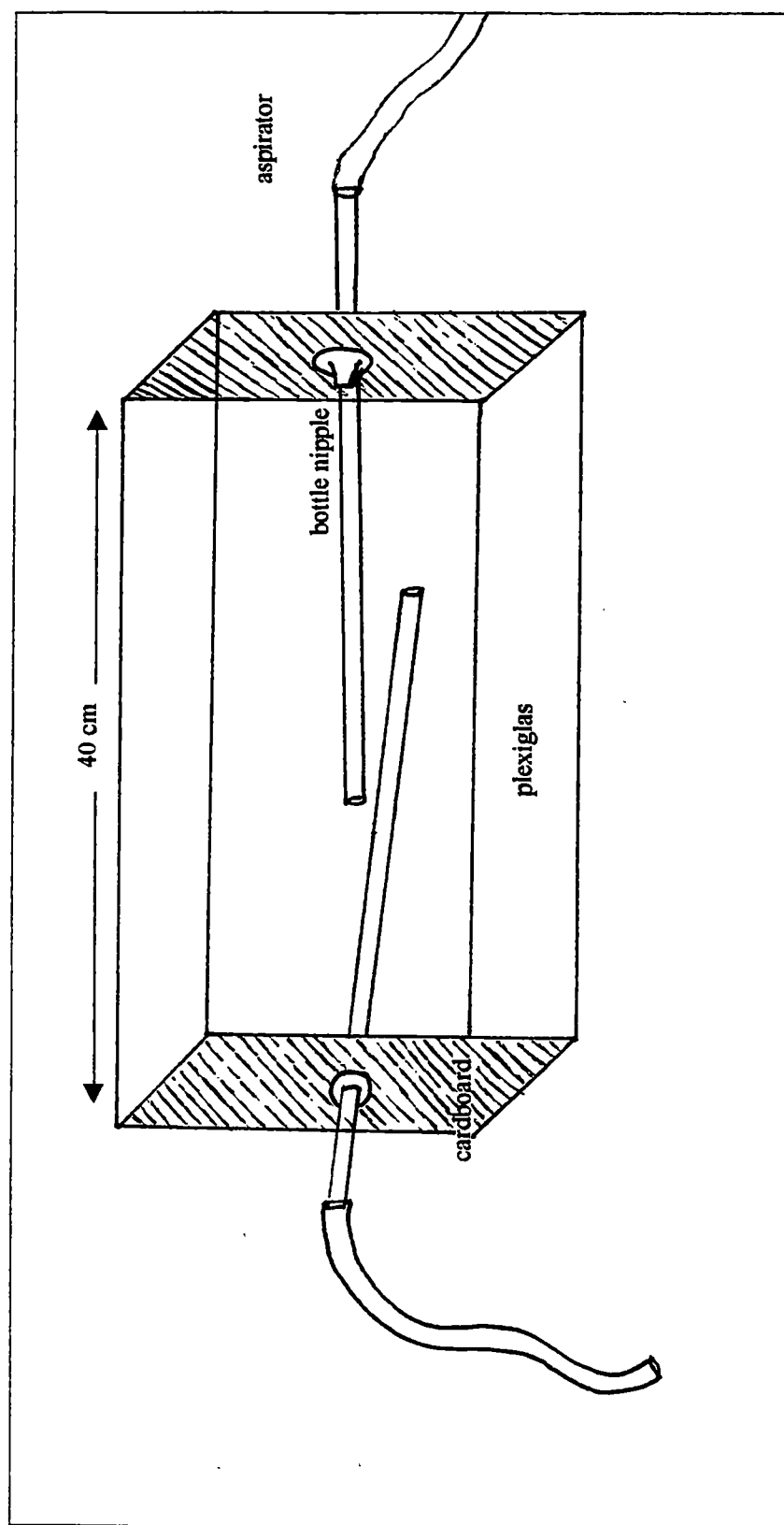


Figure 3. Testing chamber. The chamber (40 cm x 20 cm x 20 cm) was constructed of four plexiglas sides and two cardboard ends. Copulating pairs were collected during the test by inserting an aspirator through a modified bottle nipple at either end of the chamber.

length of the tests was 1 hr and they were run in the first and last 3 hr of the light cycle. I chose this time window because I generally encountered the highest number of flies at the baits in the field in the morning and in the evening. At 30 min and 45 min, I aspirated air into the chamber for 10 sec because pilot tests indicated that this increased activity levels. Copulating pairs were aspirated out of the chamber and placed into individual vials. The pairs were frozen and then dissected to determine population identity (see above).

Tests of interspecific discrimination

To test for the presence of interpopulation variation in levels of premating isolation between *D. affinis* and *D. athabasca*, I ran a series of mate choice tests between several *D. affinis* lines and a tester line of *D. athabasca* from Knoxville, TN (TN4). A subset of the lines used in the intraspecific tests were chosen for interspecific mate choice tests (Table 1, Appendix).

My choice of lines was in part determined by the results of the intraspecific tests, a percentage of which had been completed at the start of the interspecific tests. Because these results did not indicate the presence of assortative mating by any of the lines, I simply chose the lines with the lowest number designation. WIB was excluded because of its close geographic proximity to WIC. IOB was excluded because the viability of this line was declining. A total of ten *D. affinis* isofemale lines from 6 states was tested with an isofemale *D. athabasca* tester line (TN4). The testing procedures were the same as those described above for the intraspecific tests.

Statistical analyses

The results of multiple mate choice tests between two groups, A and B, can be categorized into 4 types of mating: $A♀A♂$, $A♀B♂$, $B♀A♂$, and $B♀B♂$. A higher

frequency of homotypic matings ($A♀A♂$, $B♀B♂$) indicates assortative mating and some level of isolation. The level of sexual isolation is often measured by a Joint Isolation Index (I) which is calculated by dividing the difference between homotypic and heterotypic matings by the total number of matings (Gilbert and Starmer 1985). The index is negative if heterotypic matings outnumber homotypic matings, $I=0$ if there is no isolation, and $I=1$ if isolation is complete.

For the intraspecific tests, I calculated a Joint Isolation Index (I) (Gilbert and Starmer 1985) for each replicate and for the pooled data from the 3 replicates for each test combination. I tested the significance of each I by using the formula $Z=(2\sqrt{n})(p - 0.5)$ where p is the proportion of homotypic matings and $S.E. = \sqrt{[(1-I^2)/n]}$ (Kaneshiro 1976; Markow 1981). The null hypothesis of random mating can be rejected ($p<0.01$) when $-2.53>Z>+2.53$. Due to the high number of tests, the significance level, $p<0.01$, was chosen for all statistical tests to reduce the chances of a Type I error.

The Isolation Index is sensitive to differences in mating vigor which may be affected by experimental variables (e.g. disease, culture conditions). In addition, the Isolation Index is not well suited to measuring asymmetric isolation (Hollocher et al. 1997). It has been suggested that a goodness-of-fit test may be a more appropriate test (Gilbert and Starmer 1985). Therefore, a heterogeneity G-test (Sokal and Rohlf 1995) was also performed to test the null hypothesis of random mating with an expected ratio of 1:1:1:1 in the 4 possible pair combinations ($A♀A♂$, $A♀B♂$, $B♀A♂$, and $B♀B♂$). The G-test also provides an estimate of the heterogeneity of the replicates. A second G-test was used to test for deviation from an expected 1:1 ratio in the number of mated males and females of the two lines in each test ($A♀:B♀$, $A♂:B♂$). Significant results in the

second G-test help to discern when significant results in the first G-test may be due to differences in mating propensity or vigor between males of the tested line and the tester line.

A Joint Isolation Index (see above) was also calculated for each replicate and the pooled results of the interspecific tests. The G-test could not be used to test for a deviation from an expected 1:1:1:1 ratio of matings in the four types of pairings because of the presence of 0's. A G-test was used to test for differences in mating vigor between *D. affinis* and *D. athabasca* individuals in each test.

II. RESULTS

Intraspecific tests

The percentage of flies mating in each test ranged from 21% to 61% ($\bar{x} \pm \text{SE}$: 40.20 ± 1.17). One line (WIC42) had an extremely low number of matings in all three replicates (Table 2, Appendix) and also had a very low number of matings in the interspecific tests. In addition, offspring production by this line was unusually low. Therefore WIC42 will be omitted from the following discussion of the test results.

Based on the Isolation Index values, only two of the 21 tested lines (ILA7 and VAC11) exhibit significant isolation with respect to the tested line (TN2) (Table 2, Appendix) and the estimated levels of isolation are low in both cases. A significant level of symmetric isolation ($I=0.29$, $p<0.01$) was found between ILA7 and TN2 only when the replicate data were pooled. In all three VAC11 replicates, a higher proportion of VAC11 females mated homotypically, although the I value was significant for only one replicate ($I=0.38$, $p<0.01$) and for the pooled results ($I=0.27$, $p<0.01$). The low level of isolation is asymmetric; TN2 females were involved in approximately equal numbers of homotypic and heterotypic matings.

The heterogeneity G-test revealed that all replicate results within each line combination were homogeneous ($p<0.01$) with the exception of WIB4. The heterogeneity of this test combination was due to a high proportion of homotypic matings by WIB1 females in WIB1-1 and a high proportion of heterotypic matings in WIB1-2.

Mating was nonrandom in a total of eight individual replicates and the pooled results from three of the line combinations as indicated by a significant G-statistic ($p<0.01$) (Table 3, Appendix: lists only the line combinations which had a significant

result). The biological meaning of the assortative mating found in VAC5-1 and WIB4-2 is unclear because the pattern was not found in the two other replicates of the line combinations (Table 2, Appendix).

Significant nonrandom mating was found in several tests which did not have significant Isolation Indices (Table 2 & 3, Appendix). The nonrandom mating patterns of SDA13-1, SDA18-3, and VAC1-2 appear to be due to significant differences in mating vigor between males of the tested and tester lines (Table 2 & 3, Appendix). A nonrandom mating pattern was also found in ILA2-3 and SDA13-3.

SDA18-pooled and VAC11-pooled are the final two tests in which the mating pattern was significantly nonrandom. This is concordant with the isolation estimates (I) which was statistically significant for VAC11 and approached significance for SDA18, although in both cases the isolation level was low (Table 2, Appendix).

Interspecific Tests

The percentage of flies mating in the interspecific tests ranged from 5% to 46% ($\bar{x} \pm \text{SE}$: 32.77 ± 1.68). A high level of isolation ($I > 0.71$) between the *D. affinis* lines and the tester line of *D. athabasca* ($A \text{♀} B \text{♂}$ and $B \text{♀} A \text{♂}$) was found in all tests except WIC42 (as I noted above, WIC42 is excluded from discussion due to low viability) (Table 4, Appendix). The lowest levels of isolation were found in the WIC3 tests (Table 4, Appendix). In WIC3-2 and WIC3-3 approximately half of the *D. athabasca* females mated with *D. affinis* males ($B \text{♀} A \text{♂}$). The *D. affinis* females were more discriminating; no *D. affinis* females mated with *D. athabasca* males ($A \text{♀} B \text{♂}$). The second highest proportion of interspecific matings occurred in PA-2 where 5 of 21 matings involving *D.*

athabasca females were heterotypic. In the other two PA replicates no *D. athabasca* females mated with *D. affinis* males.

The proportion of interspecific matings was very low in the remainder of the tests (Table 4). When the results from all tested line combinations are pooled, there were totals of 572 A♀A♂ matings, 11 A♀B♂ matings, 29 B♀A♂ matings, and 371 B♀B♂ matings. The G-test results indicate a significant difference in mating propensity in several tests (Table 5, Appendix). In the majority of these tests, the number of mated male and female *D. affinis* was significantly higher than the number of mated *D. athabasca* individuals (Table 5, Appendix).

IV. DISCUSSION

Intraspecific discrimination

Geographic variation in mating behavior has been reported for the majority of species that have been tested (reviewed by Butlin 1995a). Because assortative mating often occurs between populations that exhibit little morphological or genetic differences, mating signal and preference evolution may be rapid relative to the evolution of other characters (Wells and Henry 1998). To determine whether geographic variation is a general and common phenomenon, additional case studies are required.

The results of this study suggest that there is little biologically significant variation in mating behavior between *Drosophila affinis* populations. A significant level of isolation was found between the tested and tester lines in only two of 21 combinations. In ILA7 and VAC11 the level of isolation was low ($I=0.29$, $I=0.27$). A significant level of isolation was not found in the three other lines from ILA and VAC, which suggests there may be variation in mating behavior within these populations.

Significant nonrandom mating, as indicated by a significant G-statistic, was found in several tests which did not have significant Isolation Indices (Table 2 & 3, Appendix). In most of the tests in which mating patterns were significantly nonrandom, there were significant differences in the numbers of males mating from the tested line and the TN2 line. In the lines without statistically significant differences in the number of males or females mating, inspection of the data suggests that the significant deviation from random mating may be due to the combined effect of a lower number of both TN2 males and females involved in mating.

It is difficult to interpret the biological meaning of tests in which there are nonrandom mating patterns but no significant levels of isolation as indicated by a significant Isolation Index.. A higher number of females from both lines may mate with a male from a single line because they are exercising a preference or because males of that line are more likely to engage in courtship. Without detailed behavioral observations or a different mate choice test design, it is impossible to determine whether the nonrandom mating pattern is due to female choice or vigor differences between the two lines. When considering reproductive isolation, the more conservative assumption is that nonrandom mating is due to vigor effects.

The significant G-statistics for SDA-18 pooled and VAC11-pooled are the only tests in which the nonrandom mating can safely be interpreted as a result of low levels of isolation because the I value was statistically significant for VAC11 and approached significance for SDA18.

Comparisons of patterns of geographic variation in different species may be a viable approach to identifying the most critical factors influencing the evolutionary rate and interpopulation divergence of courtship behavior. The majority of published studies that have considered geographic variation in mating behavior in *Drosophila* species have reported the presence of assortative mating between populations (reviewed by Butlin 1995a). One of the most interesting cases of geographic variation is found in *D. athabasca*, a close relative of *D. affinis*. The levels of isolation between the *D. athabasca* populations ($I=0.6-0.9$) are among the highest relative to the levels of genetic divergence reported for *Drosophila* (Coyne and Orr 1989). The presence of extensive variation in *D. athabasca* and the lack of such variation in *D. affinis* within the same

geographic area is interesting because of their phylogenetic affinity and the similarity of their courtship and ecology. Below I discuss possible explanations for the different levels of geographic variation in mating behavior found in *D. affinis* and *D. athabasca*.

One physiological characteristic that may be responsible for the different patterns of variation found in the two species is tolerance to extreme temperature and desiccation. *Drosophila affinis* can withstand both higher temperatures and lower levels of humidity than *D. athabasca* (Richmond 1980; Fogleman 1982). These laboratory findings are concordant with documented shifts in relative frequencies of the two species at different elevations and at different times of the year (Miller and Weeks 1964; Richmond 1980). If *D. athabasca* is less able to withstand environmental conditions between "islands" of their preferred woodland habitat, gene flow between *D. athabasca* populations is likely to be reduced. This would allow independent evolution of mating signals and preferences in disjunct populations leading to the extensive level of geographic variation that has been documented. Because *D. affinis* has a greater ability to withstand higher temperatures and lower levels of humidity, a higher rate of migration between *D. affinis* populations may prohibit independent divergent evolution.

Ford and Aquadro (1996) proposed that range expansion after the last ice age led to disruptive natural selection between the western-northern semispecies and the ancestor of the two eastern semispecies of *D. athabasca*, resulting in selection for behavioral isolation between the two types. The isolation between the two eastern *D. athabasca* semispecies may be a result of a selective sweep that occurred in the last 20,000 years in an isolated southern population, a scenario that is consistent with molecular data (Ford and Aquadro 1996). Ford and Aquadro (1996) admit these ideas are speculative and

require knowledge of adaptive differences between the semispecies and the genetic basis of their courtship behavior. If such information about *D. athabasca* was available, it would be worthwhile to consider the same factors in *D. affinis*.

Intersexual selection is the primary force thought to be responsible for rapid interpopulation divergence in mating signals and preferences. If so, weaker female preferences or reduced opportunities for females to exercise preferences would then decrease the probability of the evolution of geographic variation in mating behavior. For example, a reduced opportunity for female choice due to a high incidence of sneaky mating has been used to explain the lack of reproductive isolation between Trinidadian guppy (*Poecilia reticulata*) populations that have diverged rapidly in ecological characters (Magurran 1998). Based on my behavioral observations, forced copulations are unlikely to occur in *D. affinis*, but there are other factors that may result in reduced opportunity for female choice in this species. Because sexual selection is thought to increase rates of divergence, it would be worthwhile to determine if there are differences between *D. athabasca* and *D. affinis* in parameters related to sexual selection.

Although the populations that were included in this study represent a significant portion of the range of *D. affinis*, populations from the northeastern and southeastern limits of its range were not included. Therefore, it may be hasty to conclude that there is a complete absence of variation across its entire range. For example, no assortative mating was detected in 38 interpopulation crosses representing 29 worldwide populations of *D. melanogaster* (Henderson & Lambert 1982). A later study then documented the presence of strong isolation between populations from Zimbabwe and several other populations (Wu et al. 1995).

This study was designed to test for reproductive isolation between *D. affinis* populations due to the presence of premating barriers. One of the critical issues in the study of speciation is the rate of the evolution of premating isolation relative to the rate of the evolution of postmating isolation (Coyne & Orr 1989). Although there are no significant levels of premating isolation between *D. affinis* populations, there is the possibility that some form of postmating isolation may exist between among the populations due to hybrid sterility or inviability. Testing this possibility would be a valuable area for future investigation but may require time intensive breeding designs.

Interspecific discrimination

The importance of reinforcement in the enhancement of reproductive isolation has been intensely debated but some data support its plausibility in both insects and frogs (Coyne and Orr 1989; Noor 1995; Littlejohn 1999). Traditional models of reinforcement predict divergence in female preferences between nascent species which leads to assortative mating between populations that are either sympatric or allopatric with respect to the closely related species. Another possible outcome of reinforcement is an increase in the level of female mating discrimination without divergence in the preference or signal; this process has been coined the “discrimination enhancement” model of reinforcement whereas the traditional model of reinforcement is referred to as the “preference evolution” model (Gwynne and Morris 1986; Kelly and Noor 1996).

The results of the intraspecific mate choice tests of this study indicate that there has been no divergence of mating signals and preferences between *D. affinis* populations, ruling out the possibility that reinforcement through preference evolution has occurred. Interspecific mate choice tests were carried out to test for geographic

variation in discrimination by *D. affinis* females against *D. athabasca* males, which may result from “discrimination enhancement.” Such variation between *D. affinis* populations might be expected because populations are likely to be sympatric with different *D. athabasca* semispecies that differ in mating signals or they may encounter *D. athabasca* at significantly different rates.

There is no evidence of geographic variation in interspecific mate discrimination between the *D. affinis* populations included in this study. The estimates of isolation between the *D. affinis* lines and the *D. athabasca* tester line (TN4) were all high, with the majority having I values above .9. One cross had a relatively high proportion of heterospecific matings: in two WIC3 replicates, almost half of the *D. athabasca* females mated with *D. affinis* males. If this were due to a difference in the mating signals of WIC3 males, there would have presumably been assortative mating between WIC3 and the *D. affinis* tester line (TN2). This was not the case, so the reason for the increased proportion of heterospecific matings in the WIC3 tests is unclear.

The levels of isolation between *D. affinis* and *D. athabasca* found in this study differ from those reported by Miller (1958). He found a high frequency of mating between *D. affinis* females and *D. athabasca* males (90/136) and a total lack of matings between *D. athabasca* females and *D. affinis* males (0/126) in 10 day no choice tests. Miller (1958) used *D. athabasca* collected from the western United States and a mutant strain of *D. affinis* (geographic origin unknown) which may explain why he found different levels of interspecific matings. It is likely that the different test designs (multiple choice vs. no choice) would result in different estimates of isolation (Markow 1981) but this does not explain why estimates for the reciprocal crosses would differ in

two different directions. A higher estimate of isolation is likely in multiple choice tests so the lower isolation estimate of this study between *D. athabasca* females and *D. affinis* males is unexpected. Only one line of *D. athabasca* was used in this study so it may be that females of this line are unique in their lower levels of interspecific discrimination.

Conclusions

Drosophila affinis is one of the few members of its genus for which a relative lack of geographic variation in mating behavior has been documented. This lack of variation suggests that the opposing models of mating behavior evolution (stability vs. lability) may both be valid, depending on the taxa considered. Although the results of this study lend support to the stability model, it may be that the process(es) responsible for the lack of variation in *D. affinis* differ from that used as the foundation for the stability model. Further investigation is necessary to strengthen the conclusion that the stability model appropriately describes the evolution of mating behavior in *D. affinis*. For example, knowledge of the nature of selection (directional vs. stabilizing) on mating behavior, the amount of genetic variation in both signals and preferences, and measures of the extent of gene flow between populations would be necessary to strengthen conclusions about the evolution of mating behavior in *D. affinis*.

The presence and lack of extensive interpopulation divergence within *D. athabasca* and *D. affinis*, respectively, suggests that subtle differences among species may be important in determining patterns of geographic variation in mating signals and preferences. Identification of those differences would be a valuable contribution to our understanding of the evolution of mating behavior and the origins of reproductive isolation.

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APPENDIX

Table 2. Estimates of premating isolation between *Drosophila affinis* populations. Multiple choice tests were run between tested lines (A) and a tester line from Tennessee (B). The level of isolation is measured by the Joint Isolation Index (I) which is significant ($p < .01$) when $-2.53 > Z > +2.53$. See Table 1 for collection sites of the tested lines.

Tested Line	Test	A♀A♂	A♀B♂	B♀A♂	B♀B♂	I±S.E.	Z
ILA2	1	9	9	13	10	-0.07±0.16	-0.47
	2	14	7	9	17	0.32±0.12	2.18
	3	18	7	6	3	0.24±0.15	1.37
	Pooled	41	23	28	30	0.16±0.01	1.8
ILA4	1	12	18	6	15	0.06±0.14	0.42
	2	8	7	6	14	0.26±0.15	1.52
	3	6	7	10	13	0.06±0.16	0.33
	Pooled	26	32	22	42	0.11±0.01	1.27
ILA7	1	13	7	2	9	0.42±0.14	2.33
	2	14	7	10	16	0.28±0.13	1.9
	3	5	5	6	10	0.15±0.18	0.78
	Pooled	32	19	18	35	0.29±0.01	2.94*
ILA9	1	13	11	10	11	0.07±0.14	0.45
	2	11	11	7	19	0.25±0.13	1.7
	3	15	7	11	7	0.1±0.15	0.63
	Pooled	39	29	28	37	0.14±0.01	1.65
IOB2	1	8	11	11	13	-0.02±0.15	-0.15
	2	11	8	4	11	0.29±0.14	1.7
	3	15	8	13	14	0.16±0.13	1.13
	Pooled	34	27	28	38	0.13±0.01	1.5
PA	1	20	12	12	12	0.14±0.12	1.06
	2	19	8	15	8	0.08±0.14	0.57
	3	11	11	11	18	0.14±0.13	0.98
	Pooled	50	31	38	38	0.12±0.01	1.52
SDA1	1	19	5	12	6	0.19±0.14	1.23
	2	9	15	11	16	-0.02±0.14	-0.14
	3	7	4	7	11	0.24±0.16	1.3
	Pooled	35	24	30	33	0.11±0.01	1.27
SDA13	1	2	7	5	15	0.17±0.17	0.93
	2	5	6	9	17	0.19±0.15	1.15
	3	13	5	11	5	0.06±0.17	0.34
	Pooled	20	18	25	37	0.14±0.01	1.4

* $p < .01$

Table 2. (continued)

Tested Line	Test	A♀A♂	A♀B♂	B♀A♂	B♀B♂	I±S.E.	Z
SDA18	1	14	13	4	13	0.23±0.13	1.5
	2	11	4	7	7	0.24±0.16	1.3
	3	19	1	13	4	0.24±0.14	1.48
	Pooled	44	18	24	24	0.24±0.01	2.48
SDA19	1	11	9	11	5	-0.11±0.17	-0.67
	2	12	4	9	6	0.16±0.16	0.9
	3	14	9	17	6	-0.13±0.16	-0.88
	Pooled	37	22	37	17	-0.04±0.01	-0.47
SDA21	1	10	16	12	19	0.017±0.13	0.13
	2	16	6	13	12	0.19±0.13	1.31
	3	17	5	14	9	0.16±0.14	1.04
	Pooled	43	27	39	40	0.11±0.01	1.39
TN5	1	8	14	3	13	0.11±0.15	0.65
	2	12	10	12	8	-0.05±0.16	-0.3
	3	15	14	10	17	0.14±0.12	1.06
	Pooled	35	38	25	38	0.07±0.01	0.86
VAC1	1	13	6	13	13	0.15±0.14	1.04
	2	14	3	20	6	-0.07±0.16	-0.46
	3	10	7	18	8	-0.016±0.16	-0.106
	Pooled	37	16	51	27	-0.02±0.01	-0.26
VAC11	1	26	6	13	16	0.38±0.10	2.94*
	2	20	10	12	11	0.17±0.12	1.23
	3	18	8	8	9	0.26±0.13	1.67
	Pooled	64	24	33	36	0.27±0.01	3.43*
VAC12	1	9	9	9	1	-0.9±0.21	-1.51
	2	11	14	10	18	0.09±0.13	0.69
	3	8	10	16	10	-0.18±0.16	-1.21
	Pooled	28	33	35	29	-0.09±0.01	-0.98
VAC5	1	10	0	13	7	0.13±0.17	0.73
	2	8	9	14	8	-0.18±0.17	-1.12
	3	10	7	18	8	0.31±0.14	1.86
	Pooled	32	13	35	24	0.08±0.01	0.78

*p<.01

Table 2. (continued)

Tested Line	Test	A♀A♂	A♀B♂	B♀A♂	B♀B♂	I±S.E.	Z
WIB1	1	15	10	6	9	0.2±0.14	1.26
	2	14	8	10	9	0.12±0.14	0.78
	3	12	14	10	10	-0.04±0.15	-0.29
	Pooled	41	32	26	28	0.09±0.01	0.98
WIB4	1	12	6	1	7	0.46±0.14	2.35
	2	1	10	8	13	-0.13±0.19	-0.71
	3	10	12	11	13	0.0±0.15	0
	Pooled	23	28	20	33	0.08±0.01	0.78
WIB7	1	8	13	11	22	0.11±0.13	0.82
	2	7	9	4	13	0.21±0.15	1.22
	3	12	3	8	4	0.19±0.17	0.96
	Pooled	27	25	23	39	0.15±0.01	1.6
WIC3	1	7	7	8	6	-0.07±0.20	-0.38
	2	10	11	11	9	-0.07±0.16	-0.47
	3	6	7	12	6	-0.23±0.20	-1.26
	Pooled	23	25	31	21	-0.12±0.01	-1.2
WIC42	1	0	0	0	21	1±0.00	4.58*
	2	3	0	0	22	1±0.00	5*
	3	4	0	0	26	1±0.00	5.48*
	Pooled	7	0	0	69	1±0.00	16.45*

*p<.01

Table 3. Tests for nonrandom mating and differences in mating propensity between *Drosophila affinis* lines. Multiple choice tests were run between tested lines (A) and a tester line from Tennessee (B). Heterogeneity G-tests were used to test for deviation from random mating (1:1:1:1 ratio between A♀A♂:A♀B♂: B♀A♂: B♀B♂) and a difference in mating propensity between the tester and tested line females and males (1:1 ratio between A♀:B♀ and A♂:B♂). A significant G_H indicates the replicate results were heterogeneous. Line combinations without any significant results are not listed.

Tested Line (A)	Test	Test for random mating	Differences in mating propensity	
		A♀A♂:A♀B♂:B♀A♂:B♀B♂	A♀:B♀	A♂:B♂
ILA2	1			
	2			
	3	*		
	Pooled G_H			
SDA13	1	*		<*
	2			
	3	*		
	Pooled G_H			
SDA18	1			
	2			
	3	*		>*
	Pooled G_H	*		
SDA19	1			
	2			
	3			
	Pooled G_H			>*
VAC1	1			
	2	*		>*
	3			
	Pooled G_H	*		>*
VAC5	1	*		>*
	2			
	3			
	Pooled G_H			>*

* $p < .01$

<> indicate direction of inequality

Table 3. (continued)

Tested Line (A)	Test	Test for random mating		Differences in mating propensity	
		A♀A♂:A♀B♂:B♀A♂:B♀B♂		A♀:B♀	A♂:B♂
VAC11	1		*		
	2				
	3				
	Pooled		*		>*
	G _H				
WIB4	1				
	2		*		
	3				
	Pooled				
	G _H		*		
WIB7	1				
	2				
	3				
	Pooled				
	G _H				*
WIC42	1			<*	<*
	2			<*	<*
	3			<*	<*
	Pooled		*	<*	<*
	G _H				

*p<.01

<> indicate the direction of the inequality

Table 4. Estimates of isolation between *Drosophila affinis* and *D. athabasca*. Multiple choice tests were run between *D. affinis* lines (A) from different geographic locations and a tester line of *D. athabasca* from TN (B). The level of isolation is measured by the Joint Isolation Index (I) which is significant ($p < .01$) when $-2.53 > Z > +2.53$.

Tested Line	Test	A♀A♂	A♀B♂	B♀A♂	B♀B♂	I	Z
ILA2	1	20	0	0	13	1±0.00	5.74*
	2	15	0	0	19	1±0.00	5.83*
	3	18	0	1	8	0.92±0.05	4.80*
	Pooled	53	0	1	40	0.98±0.00	9.48*
ILA4	1	11	0	0	8	1±0.00	4.35*
	2	23	0	0	10	1±0.00	5.74*
	3	15	0	0	12	1±0.00	5.20*
	Pooled	49	0	0	30	1±0.00	8.89*
PA	1	26	0	0	20	1±0.00	6.78*
	2	24	0	5	16	0.77±0.07	5.21*
	3	19	1	0	14	0.94±0.04	5.48*
	Pooled	69	1	5	50	0.90±0.00	10.10*
SDA1	1	21	3	1	12	0.78±0.08	4.76*
	2	20	0	2	12	0.88±0.06	5.14*
	3	15	0	0	7	1±0.00	4.69*
	Pooled	56	3	3	31	0.87±0.00	8.39*
SDA13	1	20	0	0	9	1±0.00	5.38*
	2	14	1	0	18	0.93±0.04	5.40*
	3	18	1	2	18	0.85±0.06	5.28*
	Pooled	52	2	2	45	0.92±0.00	9.25*
TN2	1	26	1	0	14	0.95±0.03	6.09*
	2	19	3	0	22	0.86±0.06	5.72*
	3	17	0	0	19	1±0.00	6.00*
	Pooled	62	4	0	55	0.93±0.00	10.27*
VAC1	1	27	0	0	8	1±0.00	5.91*
	2	24	0	1	19	0.94±0.03	6.33*
	3	26	0	1	13	0.95±0.04	6.00*
	Pooled	77	0	2	40	0.97±0.00	10.50*

* $p < .01$

Table 4. (continued)

Tested Line	Test	A♀A♂	A♀B♂	B♀A♂	B♀B♂	I	Z
WIC3	1	23	0	0	8	1±0.00	5.56*
	2	21	0	5	9	0.71±0.09	4.22*
	3	31	0	4	8	0.81±0.07	5.33*
	Pooled	75	0	9	25	0.83±0.00	8.71*
WIC42	1	11	0	0	12	1±0.00	4.79*
	2	1	0	2	2	0.2±0.4	0.44
	3	3	0	0	14	1±0.00	4.12*
	Pooled	15	0	2	28	0.91±0.01	6.11*

*p<.01

Table 5. Tests for differences in mating propensity between *Drosophila affinis* and *D. athabasca*. Multiple choice tests were run between *D. affinis* lines (A) from different geographic locations and a tester line of *D. athabasca* from TN (B). Heterogeneity G-tests were used to test for differences in mating propensity or vigor between the tested line and the tester line. All replicates within each test combination were homogeneous (no significant G_H estimates). Only line combinations with significant results are listed.

Tested Line (A)	Test	A♀:B♀	A♂:B♂
SDA1	1		
	2		
	3		
	Pooled	>*	>*
VAC1	1	>*	>*
	2		
	3		
	Pooled	>*	>*
VAC5	1		
	2		>*
	3		
	Pooled	>*	>*
WIC3	1	>*	>*
	2		>*
	3	>*	>*
	Pooled	>*	>*
WIC42	1		
	2		
	3	<*	<*
	Pooled		

* $p < .01$

<> indicate the direction of the inequality

VITA

Jessica Bier was born on April 8, 1972 in Durham, North Carolina. She lived in Florida and New York before her parents settled permanently in Ferrum, Virginia. She attended Juniata College in Pennsylvania for one year and then finished a degree in Environmental Science and Philosophy at Ferrum College in 1994. She has worked as a certified nursing assistant, archaeological field technician, library assistant, gypsy moth trapper, graduate teaching assistant, and is currently a fire effects monitor with the NPS. She entered the Department of Ecology and Evolutionary Biology, UTK, in 1995.