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Desiccation survival traits in natural populations of two siblings species of the genus *Drosophila*

Laverde, Michael Joseph, Ph.D.

City University of New York, 1995

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DESICCATION SURVIVAL TRAITS IN NATURAL POPULATIONS OF TWO SIBLING SPECIES OF THE GENUS *DROSOPHILA*

by

MICHAEL J. LAVERDE

A dissertation submitted to the Graduate Faculty in Biology in partial fulfillment of the requirements for the degree of Doctor of Philosophy, The City University of New York

1995

This manuscript has been read and accepted for the Graduate Faculty in Biology in satisfaction of the dissertation requirement for the degree of Doctor of Philosophy.

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ABSTRACT

DESICCATION SURVIVAL TRAITS IN NATURAL POPULATIONS OF TWO SIBLING SPECIES OF THE GENUS *DROSOPHILA*

by

MICHAEL J. LAVERDE

Advisers: Professor Louis Levine
Professor Robert F. Rockwell

Desiccation is a primary problem for all land insects. This thesis project investigated the question: What is the relationship between the traits of desiccation resistance, vagility, and body size?

Many traits may contribute to survival in a desiccating environment. This study focused on desiccation resistance (survival), vagility (movement away from a central area), and body size. A comparative examination of these traits over time for the sibling species *Drosophila melanogaster* and *D. simulans* was performed. These have been tested for desiccation resistance at four time periods (4, 8, 12, and 24 hours), for adult vagility in the Sakai double-ringed system at two time periods (4 and 24 hours), and for body size estimated from the length of the III longitudinal vein in the right wing. These measurements have been taken over a two year period (two seasons) on four collections. Annual, seasonal, species, and sex differences and changes, as well as expressed genotypic variances and patterns of correlation were investigated.

Generally *D. melanogaster* is more resistant to desiccation and larger than *D. simulans*, possibly representing different evolutionary histories for the species. Females were found generally more resistant to desiccation than their male counterparts in both species, and have a larger body size. The larger females may have more fat and internal water storage allowing them to resist desiccation better than the males in order to ensure successful depositing of their eggs. No annual, seasonal, or temporal changes were detected. Very little, if any, expressed genetic variance was found for any of the traits between the two species and between the sexes. The genetic architecture of these populations may have been modified by natural selection such that the genetic variance is not expressed as phenotypic differences, or have been reduced. Desiccation resistance was found to be linked with a greater vagility and a smaller body size. A lower vagility for less desiccation resistant flies may act to keep them in a suitable microhabitat. Desiccation resistance, vagility, and body size may be just three of many traits that contribute to a complex phenotype allowing greater survival with respect to desiccation stress.

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INTRODUCTION

An organism does not often use a single trait to survive a stressful condition. Rather, it makes use of several traits which together may confer an adaptive advantage to the organism for a specific environmental problem.

A serious problem for all land insects is desiccation (Bateman, 1975). (Insects, with their small size, have a large surface area to volume ratio.) Besides the insects' necessities which include the reproductive process (finding a mate, laying eggs, etc.) and obtaining food, insects must avoid desiccating. For desiccation resistance, interspecific differences have been found in *Formica* spp. and *Glossina* spp. (Bursell, 1964). Also intraspecific variation has been found in *Aedes aegypti* and *A. atrophalpus* (Bursell, 1964), and *Drosophila melanogaster* (Parsons, 1970). *D. melanogaster* has been demonstrated to show preferences for certain humidity ranges (Perttunen and Salmi, 1956), and it was found that different genotypes of *D. melanogaster* may exhibit different humidity preferences (Waddington *et al.*, 1954; Perttunen and Salmi, 1956).

Water loss is principally regulated either by closing of the spiracles (Fairbanks and Burch, 1970; Arlian and Eckstrand, 1975; Eckstrand, 1981) and/or behavior (Eckstrand and Richardson, 1980). Traits that allow insects to survive longer in a desiccating environment include various behavioral traits (microhabitat selection, photobehavior, vagility, etc.), morphological traits (a waxy cuticle, body size, spiracular control, etc.), and physiological traits (fat storage, metabolism, etc.) (Ringo and Wood, 1984).

For *Drosophila*, adaptations related to water balance are of the utmost importance because they lose water even when placed in an atmosphere that contains 99% relative humidity. These flies must ingest high-water content foods or drink large amounts of water to maintain their water balance (Arlian and Eckstrand, 1975), or close their spiracles (Fairbanks and Burch, 1970).

Regulation of water loss is critical to prolonging survival time in a desiccating environment (Eckstrand and Richardson, 1980; Eckstrand, 1981). Species differences in desiccation resistance have been demonstrated (Parsons, 1970; McKenzie and Parsons, 1974; Bateman, 1975). These investigations also found that female flies survive desiccating conditions better than the male flies. Selection for increased resistance to desiccation also has been demonstrated (Ringo and Wood, 1984). This ability, and, thus, survival under desiccation stress, varies within, as well as between, species of *Drosophila* (Levins, 1969; McKenzie and Parsons, 1974; Eckstrand and Richardson, 1980; Eckstrand, 1981).

According to Pittendrigh (1958), a critical factor in determining distribution within the genus *Drosophila* may be the extent to which species can prevent water loss. The pressure to maintain water balance may affect the flies' movements (Arlian and Eckstrand, 1975). Since flies feed shortly after sunrise and before sunset, when humidity is high, selection should favor individual flies that confine their feeding activity to those times of the day. Also, low relative humidity and limited water should exert considerable selective pressure to travel to a suitable microhabitat (Arlian and Eckstrand, 1975). The ability to move not only depends on the genetic composition of these emigrating flies, but also on their propensity to move—their vagility.

Vagility is the ability of an organism to move from one place to another (Sakai *et al.*, 1958). The testing of vagility in natural populations of *Drosophila* is not abundant. Previous investigations have searched the effects of genotypes and environments to discover the norm of reaction (Tantawy *et al.*, 1975; Rockwell *et al.*, 1978; Mikasa and Narise, 1983). The propensity to move may be driven by genetic factors, by environmental factors, or by the interaction of both factors.

A trait that allows an organism to adapt to one problem may also be useful in another situation especially when combined with other traits. Adaptation is rarely a matter of modifying one or two traits; rather, it normally involves the development of complex adaptive strategies composed of arrays of interacting mechanisms (Lynch *et al.*, 1988). Generally, these traits are those for which the genetic control is polygenic: foraging behavior (Pyke *et al.*, 1977), mating preferences (Lande, 1981), life history strategies (Stearns, 1977), and body size (Atchley and Cowley, 1981).

While it is the phenotype which confers adaptation, it is the genotype which is passed on. Although only heritable traits can respond to selection, there are two complications: genotype-by-environment interactions and additive genetic correlations (genetic coupling of relevant phenotypes) between traits (Lynch *et al.*, 1988). Phenotypes and genotypes are not always linearly related. Phenotypic flexibility, reflecting genotype-by-environment interactions, may be a byproduct of the "adaptive nature" of some traits (e.g., behaviors). According to Lynch *et al.* (1988), what is ecologically reasonable is also genetically feasible.

Population geneticists have considered genes as separate entities, but natural populations are far more complicated. Two factors—linkage and interactions between genes—cause the consideration of the genotype as a whole (Anderson, 1968). In a population, the genes may be selected to operate together to confer maximal fitness (Anderson, 1968). Internal adjustment coadaptation (Dobzhansky, 1949)—adjustment of a collection of genes in the population—allows the establishment of favorable linkages by the selection of genes which interact epistatically for increased adaptation and reproduction. Anderson (1968) found natural populations of *Drosophila pseudoobscura* and *D. subobscura* possessed coadapted genetic systems with genes mutually adjusted by selection for favorable interactions.

Understanding coadaptation requires the examination of phenotypes and genotypes. Many traits may contribute to survival with respect to desiccation stress to form a complex phenotype which has physiological, behavioral, and morphological, components (Lynch *et al.*, 1988). This study focused on desiccation resistance (survival), vagility (movement away from a central area), and body size. Genetic correlations between these traits may contribute to a single complex phenotype.

Since desiccation survival is a primary concern for *Drosophila*, this thesis project asked: What is the interaction among the traits of desiccation resistance, vagility, and body size? Is desiccation resistance a function of body size? Is desiccation linked with dispersal? This project involved the comparative examination of these traits over time for the sibling species *Drosophila melanogaster* and *D. simulans*. (These species are cosmopolitan in distribution and commensal with humans, and they have received wide attention from geneticists and ecologists). Since sibling species are morphologically and phylogenetically similar, but genetically and chromosomally distinct, a concordant relationship among these traits would demonstrate their group importance to desiccation survival. Sibling species have been the study of many population geneticists in determining their physiology (McKenzie and Parsons, 1974), behavior (Sokolowski and Hansell, 1983; Soliman and Knight, 1984; Hoffmann, 1987), lifespan (Schnebel and Grossfield, 1983), genetic architecture (Choudhary and Singh, 1987), and biochemistry (Hubby and Throckmorton, 1968).

Sibling species are similar enough to potentially display such concordance, but not so distinct as to trivialize the existence of this concordance. Assuming an underlying importance to desiccation survival of these traits, concordance would be expected in changes over time in similar species. Monitoring these grouped traits over time should allow inferences to be made about their adaptive role in regulating water loss and, therefore, extending survival time with respect to a desiccating environment. According to Lynch *et al.* (1988), comprehensive studies of these adaptive trait complexes should provide more information than from studies of isolated phenotypes.

These issues will be addressed by examining the following questions. Both means and genetic variances of the traits will be used in the evaluation.

(1) Are there annual and seasonal changes?

(2) Do the species differ?

(3) Do the sexes differ? (For desiccation resistance and body size.)

(4) If there are any changes, are they concordant across the species?

(5) What are the patterns of correlation among the traits in general and in particular with reference to any annual, seasonal, species, and sex differences?

Project:

The *Drosophila* species involved in this study are located in an area called Laguna Verde (longitude 96°24'30"W and latitude 19°43'24"N) approximately 75 km north of the city of Vera Cruz, Mexico (see Figure 1).

D. melanogaster and *D. simulans* are represented by 10 isofemale lines each and the Ives Oregon-R laboratory stock of *D. melanogaster* is used as a control or long-term laboratory stock (used to test for changes in laboratory conditions). These have been tested for desiccation resistance at four time periods (4, 8, 12, and 24 hours), and for vagility in the Sakai system (consisting of a set of interconnecting vials through which individual flies can move) at two time periods (4 and 24 hours). Body size was estimated from the length of the III longitudinal vein in the right wing (of preserved specimens). According to Levins (1969), in nature, adaptation to "physiological stresses" may be genetic, behavioral, or acclimatization; or by a combination of these.

These measurements have been taken over a two year period on four collections—which include two seasons each year. Flies were collected in the months of January and July of each year. This adds seasonal variation to the project—whether the traits are subject to seasonal or long-term changes and, if so, whether the changes occur in parallel fashion in both species. According to the 1987 weather statistics, Laguna Verde is cooler (18.8°C) and drier (0.0 mm precipitation) in the month of January, than July (27.6°C; 409 mm precip.) In environments with short-term environmental fluctuations (seasonal variations), phenotypic flexibility may be particularly important (Slodbodkin, 1968).

Each collection is designated a four digit collection number consisting of the last two digits of the year plus a two digit number representing the month. For example; flies collected during July 1988 have the collection number: 8807 and those from January, 1989: 8901. (This is year=1.) Year=2 consists of 8907 and 9001.

LITERATURE REVIEW

Desiccation:

All land organisms are affected by desiccation (Bateman, 1975). Especially for insects, with their large surface area to volume ratio. Insects must ingest high-water content foods or drink large amounts of water to maintain their water balance (Arlian and Eckstrand, 1975) in addition to their other necessities of finding food and reproducing. The spiracles must be finely controlled to allow for water conservation (Fairbanks and Burch, 1970).

For *Drosophila*, adaptations related to water balance are of the utmost importance because they lose water even when placed in an atmosphere that contains 99% relative humidity. It has also been hypothesized that the degree to which a *Drosophila* species can prevent water loss is the critical factor in determining that species' distribution (Pittendrigh, 1958).

D. melanogaster has been demonstrated to show preferences for certain humidity ranges (Perttunen and Salmi, 1956) and it was found that different genotypes of *D. melanogaster* may exhibit different humidity preferences (Waddington *et al.*, 1954; Perttunen and Salmi, 1956). Perttunen and Salmi (1956) found a preference for the dry side—avoidance of high relative humidities—in normal wild-type flies. In desiccating flies, the original preference for dry air by young flies would be reversed to a preference for moist air and the original preference for moist air by the older flies would be intensified. They found that the flies were sensitive even to small differences in humidity.

A possible neurological mechanism for the detection of humidity was investigated by Kellogg (1970). He made extracellular microelectrode recordings of the responses of thin-walled sensillae on the antennae and maxillary palpi of female *Aedes aegypti* to water vapor and carbon dioxide. One type of antennal sensilla basiconica responded to water vapor with an increase in the action potential rate and was capable of detecting a sudden increase of two percent in the relative humidity. One neuron in another type of sensilla basiconica on the maxillary palpi showed a logarithmic sensitivity to sudden increases in carbon dioxide concentration from below 0.01% to a saturation level of 0.05 to 0.5%.

In 1946, Begg and Hogben studied the humidity reactions of *D. melanogaster* (Oregon). They did not obtain any responses to humidity differences in either normal or desiccated wild-type females of this species. Males showed a response to moisture when the difference in relative humidity was greater than 50%,. The only preference for the drier side of the apparatus was shown by the antennaless mutant. They concluded that the absence of the antennae does not abolish, but modifies the humidity response (which is opposite that of normal flies). The wild-type females of *D. melanogaster* showed no response to humidity differences when desiccated, and the males only responded when the humidity difference exceeded 50% RH.

In 1952, Perttunen and Erkkilä studied the reactions of *D. melanogaster* (Berlin Wild) of mixed ages to differences in relative humidity. They found that *D. melanogaster* (Berlin Wild) differed considerably from those reported previously (Begg and Hogben, 1946) showing greater sensitivity to humidity differences in both males and females, with definite preference for drier air in a certain part of the humidity range. They found that the intensity of the humidity reaction was correlated with the degree of the higher alternative humidity available rather than with the difference in humidity. When the higher alternative humidity was between 100% and 87% RH, the normal undesiccated specimens preferred the drier alternative. When the higher alternative humidity was between 77% and 20% RH, the more moist alternative was preferred. In desiccated specimens (males 3 hours, females 5 hours, at 26°C), the original dry reaction was reversed to moist, and the original moist reaction was usually somewhat intensified. Females flies required a longer desiccation time than the males to reach the same intensity of moist reaction.

Perttunen and Syjämäki (1958) found that the normal undesiccated specimens of *D. melanogaster* showed a marked preference for the drier air (77% and 100% RH), with removal of the antennae abolishing the humidity reaction completely in undesiccated specimens. At 5 hours (24°C), desiccation reversed the dry reaction in the intact flies to a preference for moist air. A moist reaction of a much lower intensity was caused by desiccation of the antennectomized specimens (compared to the desiccated, intact specimens). Perttunen and Syjämäki concluded: "The humidity receptors presumed to mediate the reaction towards dry air must be situated on the antennae. The receptors or receptive areas mediating the response towards moist air must be present in part on the antennae, in part elsewhere in the body." Their results in which the antennae were removed from normal wild-type specimens were entirely different from those from antennaless mutants from Begg and Hogben (1946). Results obtained by Begg and Hogben in the antennaless mutant may not be necessarily applicable to the normal or antennectomized wild-type specimens of *Drosophila melanogaster*.

Fairbanks and Burch (1970) found that spiracular control is lost as flies age. In this study, they sought to determine the effect of age upon water loss of the flies and the influence of spiracular control upon such an effect by desiccating flies of different ages in atmospheres of relatively high concentrations for CO_2 and in air. They found that the age differences in selective reaction to humidity in *D. melanogaster* are due to decreased ability with age to close the spiracles. When the spiracles were kept open with CO_2 during desiccation, old flies lost no more water than young flies. The effect of age was observed only when the flies were permitted the usual control of the spiracles. The rates of water loss from flies in elevated CO_2 indicate that there is no increase in permeability of the integument with age. Great rates of water loss were associated with greater water content as seen in the young flies.

In comparing water loss (fresh body weight and non-fat wet weight changes) by desiccating differently aged adults of *D. melanogaster* in air, in CO_2 , and in a mixture, Fairbanks and Burch (1970) found that in both sexes, the rate of water loss during desiccation in air increased with age. No increase was found in the mixture. They concluded that this indicated that age-related differences in rates of water loss were due to increasing impairment of the spiracular closing mechanism with aging. According to the wet body weight data, the rate of water loss in CO_2 was higher in males than in females after exposure to the CO_2 for 90 minutes, but not for 45 minutes.

Arlian and Eckstrand (1975) conducted a study of *D. pseudoobscura* to determine: (1) the critical equilibrium activity, (2) the rates of transpiration and sorption, (3) the influence of ambient environmental water vapor activity of these rates, and (4) the metabolic rate and its contribution to water balance. They also explored the relationships between these factors and the ecology of the flies. They found that *D. pseudoobscura* Frolova lost weight at all water vapor activities below saturation, and, therefore, is not capable of active sorption of water. They found that at 0.98 and 0.50 vapor activities, the metabolic activity was the same. Results indicated that *D. pseudoobscura* maintains a water balance by regulating water loss rather than water vapor activity of the fly's environment. They postulated that transpiration rate is regulated by spiracular control. In order to maintain their water balance, these flies must drink large amounts of water or ingest food with a high-water content.

Regulation of water loss is critical to prolonging survival time in a desiccating environment (Eckstrand and Richardson, 1980; Eckstrand, 1981). Eckstrand and Richardson's overall conclusion from their studies is that desiccation is a major problem faced by *D. mimica.* In 1980, Eckstrand and Richardson studied several water balance characteristics for a strain of laboratory-reared *D. mimica* and for four populations of *D. mimica* collected at sites differing in altitude and wetness. *D. mimica,* in the absence of drinking water, were unable to maintain a water balance even in nearly saturated environments. In opposition to the idea that the size of a fly would be expected to affect desiccation resistance by way of the surface area to volume relationship, Eckstrand and Richardson found that the size of *D. mimica* was found not to be correlated with its water loss characteristics. *D. mimica* do not transpire faster as humidity decreases. They claim that neither laboratory nor field experiments support the view that large animals lose water less rapidly than small animals because of their smaller surface area to volume ratio.

These studies of Eckstrand and Richardson (1980) are in opposition to those of Parsons (1970). Parsons had found *D. melanogaster* females are more resistant to water loss than are males and that large flies have an advantage over small ones. However, body size does not correlate with hourly loss rates in *D. mimica* (Eckstrand and Richardson, 1980). Since the surface area to volume ratio says that small flies would desiccate faster than large ones, the above studies indicate that other components of water balance, such as spiracular control, metabolic control, and behavior may be important in regulating water loss.

Levins (1969) found that bigger flies (*D. melanogaster*) survived desiccation better than small ones, but that the effect is quite small. Levins measured the wings of dead and living flies separately when half the number of flies were dead when exposed to heat in a desiccation resistance experiment. However, Levins also found that the area in nature that the flies come from plays an important role. He found that his flies from cooler, higher localities were larger than those taken in the coastal hot lowlands. But when raised in the laboratory under the same conditions, the coastal flies were larger. Levins concluded that "the general observation that phenotypic differences in nature are in the same direction as the genotypic differences revealed under uniform conditions" is contradicted.

Examples of these include Tantawy and Mallah (1961) who studied *D. melanogaster* and *D. simulans* with a transect from Lebanon to Uganda along with body size increasing at each temperature for the populations from cooler climates. The same thing for *D. subobscura* (from Great Britain) was found by Prevosti (1955). Levins (1969) interpreted Tantawy and Mallah's transect by the fact that the cool areas were dry and the hot areas were wet. Levins claims that "if body size is an adaptation to desiccation stress, there will be selection for increased size in those localities where the direct action of the environment on development will also increase size." However, in Levins' own studies, the hot regions were dry. The direct effect of the environment on the developing flies is the opposite of what is expected (usually smaller flies are produced in dry areas). In this case, the direct effect of the environment is opposed by selection for greater size along the coast. However, Levins' (1969) explanation of the difference between his data and those of Tantawy and Mallah (1961) cannot be applied to the data of Prevosti (1955).

The phenomenon in which phenotypic and genotypic variation are in opposite directions was designated "countergradient variation" (Levins, 1968). According to Levins (1969), *Drosophila* may adapt to dry heat in several ways: reversible physiological acclimation, irreversible developmental flexibility, genetic variation among populations, and behavioral avoidance of the stress situations. (Levins, 1965 and 1968, give a theoretical investigation of these modes of adaptation and their interrelations.)

In accordance with the surface area to volume ratio, large flies were found to be more resistant to water loss than small flies. Since female flies are usually larger than the males of *Drosophila* species, female flies were found to be more resistant to water loss than male flies. An exception found is Eckstrand and Richardson's (1980) study. This may indicate a difference that only applies to *D. mimica.*

According to Pittendrigh (1958), a critical factor in determining distribution within the genus *Drosophila* may be the extent to which species can prevent water loss. The pressure to maintain water balance may affect the flies' movements (Arlian and Eckstrand, 1975). Since flies feed shortly after sunrise and before sunset, when humidity is high, selection should favor individual flies that confine their feeding activity to these times of the day. Those species that can better maintain their water balance in a desiccating environment should have greater opportunities to extend their distributions.

Species differences for desiccation resistance in *Drosophila* have been demonstrated by Parsons (1970), McKenzie and Parsons (1974), and Bateman (1975). This ability, and thus survival, under desiccation stress also varies within species of *Drosophila* (Levins, 1969; Parsons 1970; McKenzie and Parsons, 1974; Eckstrand and Richardson, 1980;

Eckstrand, 1981). These investigations found that female flies survive desiccating conditions better than male flies.

Pittendrigh (1958) studied the sibling species *D. pseudoobscura* and *D. persimilis* and measured water loss as percent wet-weight lost per four hours by flies maintained at 0% RH and 21°C. It was found that males lost water faster than females, and that *D. persimilis* lost water faster than *D. pseudoobscura*. He found that *D. persimilis* lost water by cuticular transpiration more rapidly than *D. pseudoobscura*, and that males lost water quicker than females.

Levins (1969) studied acclimation to dry heat—a combination of high temperature (survival at 38°C in several species of *Drosophila*) and low humidity—and found that a process of physiological acclimation tended especially to occur in broader niched species, such as *D. melanogaster*, and tended not to occur in narrow niched species, such as *D. prosaltans*. Flies were physiologically acclimated by being raised at 25°C until they were about five days old, then acclimated at 29°C for varying periods, and tested for heat resistance at 38°C. Levins showed that most of the acclimation occurred in the first 12 hours. The acclimation acquired in one day is completely lost after two days returned to 25°C (a short-term, reversible process). Therefore, he found that physiological acclimation (a physiological response for survival to dry heat) occurs rapidly (about half-completed in 12 hours), is quickly lost, and does not depend on water uptake. In broad-niched species (especially in *D. melanogaster*) adaptation to different climates depends mostly on individual flexibility and less on genetic differences among populations. None of the narrow-niched species were found to acclimate. *D. willistoni* (a moderately broad-niche species) was not found to acclimate nor show much genetic variation. Levins suggested that this species avoids desiccation stress behaviorally. Levins feels that his work may be extrapolated to the wild—such acclimation may permit feeding in dry places without immediate desiccation.

Desiccation resistance is a stress for which there is substantial variation in natural populations, which is readily relatable to habitat (Parson, 1973, 1987). When a population is exposed to stressful desiccation levels in nature, there could be rapid phenotypic and genotypic changes. Parsons (1970) found that *D. melanogaster* isofemale lines from two wild populations were shown to differ in their ability to withstand desiccation as measured by mortalities after 16 hours in a dry environment. He concluded that there are genes segregating in wild populations for ability to withstand desiccation. Strains with high wet and dry weights were shown to lose water by desiccation relatively less rapidly and have lower mortalities, than strains with lower wet and dry weights. He also studied variability within and between five inbred strains with similar results. High heritabilities for wet weight, dry weight, and mortality of 0.40, 0.41, and 0.60, respectively, were found.

McKenzie and Parsons (1974) collected populations of *D. melanogaster* and *D. simulans* from Melbourne, Vic. and Brisbance, Qld. (Australia) and examined them for resistance to desiccation. They found that the *D. melanogaster* populations was more resistant than the *D. simulans*, and that the females of each species were more resistant to desiccation than the males for both populations. *D. melanogaster*'s resistance was not effected by the change in seasons. But the resistance of *D. simulans* was, with a greater resistance to desiccation during the warmest months which became progressively less as the temperature decreased (therefore, correlated with mean temperature and temperature fluctuation) for the Melbourne environment.

McKenzie and Parsons (1974) used laboratory populations derived from founders of each area and showed similar results. Using diallel cross techniques, they found that the genetic architectures for resistance to desiccation of the Melbourne *D. melanogaster* population and of both species from Brisbance were similar—with additive, dominance, and interaction effects. They found additive, polygenic variation for desiccation resistance with no appreciable sex-linked effects. (Desiccation resistance is almost entirely additive for *D. simulans* from Melbourne.) Different genotypes appeared to be directionally selected at different times of the year. Enough variability remains to enable the population to respond to different environments as the requirements of the environment change. McKenzie and Parsons concluded that desiccation resistance is of importance to the fitness of the populations of *D. melanogaster* in Melbourne and both species in Brisbane, and of peripheral importance to the Melbourne *D. simulans* population.

Selection for increased resistance to desiccation has also been directly demonstrated. Hoffmann and Parsons (1989a) selected for increased resistance to desiccation at an 85% mortality level in *D. melanogaster* (each successive generation was based upon the 15% of flies that survived this stress). Responses to selection were rapid with the mean of three selected lines increased from 18 hours to 28 hours after nine generations of selection (based on females only). This increase exceeds the resistance of the control lines by more than 50%. The realized heritabilities were high, averaging 0.65, and are consistent with the high responses to selection. The heritabilities are substantially higher than estimates for most morphological traits and especially for conventional life-history traits. Therefore, populations have the potential to undergo extremely rapid genetic changes when exposed to dry habitats, and this is consistent with field observations.

Ringo and Wood (1984) performed a study to "measure realized heritability of desiccation resistance in *D. simulans* and to characterize the genetic and phenotypic differences between the selected and control lines." They selected for 15 generations from a genetically heterogeneous base population of *D. simulans*. Selected flies were those surviving when 50% of the initial test sample had died. Selection was successful, there being a response to selection in both sexes of both lines. F_1 and F_2 from crosses and control lines were tested for latency to immobilization in a desiccating environment with results suggesting polygenic differences between the selected and control lines with proportionate representation of sex-linked effects.

Ringo and Wood (1984) found that desiccation resistance was greater in females than in males. This desiccation resistance decreased with the age of the females. The flies became less resistant to desiccation with age, regardless of genotype or sex. In estimating water content before and after partial desiccation, they concluded that the basis of greater desiccation resistance in the selected lines, and in females compared to males, was due to a lower rate of water loss (perhaps owing to increased spiracular control). They concluded that selection may have affected spiracular closure in the desiccating environment.

Among other environmental components affecting reaction to humidity is age. Perttunen and Ahonen (1956) subjected flies to desiccation (at 26°C) and found greater weight loss in old flies than in young flies. Both the intensity and the direction of the humidity reaction were dependent on the age of the specimens. Young *D. melanogaster* of both sexes showed a strong preference for the drier alternative out of 100% and 77% RH. The intensity of the dry reaction gradually decreased during the following days, until after about two weeks the flies were virtually indifferent. Since their results show that the humidity reaction is dependent on the age of the specimens of *Drosophila*, the intensity and direction of the humidity reaction must be largely dependent on the proportion of young flies to old flies in the population. (If the majority of the flies are young, they would prefer dry. If they were old, then they were be indifferent.) Both males and females young flies showed a strong preference for the drier alternative, especially on the first day after their emergence. During the following days, the intensity of the dry reaction gradually decreases until after about two weeks the reaction is virtually indifferent. During the following weeks either the reaction remains indifferent (females), or there may be a slight preference for high humidity (males).

Stanley *et al.* (1980), in an examination of desiccation resistance in the *D. melanogaster* subgroup, studied six species for the time taken for half the flies to die (LT_{50}) when maintained at 0% RH and 25°C. The survivorship of *D. melanogaster* was highest and that of *D. erecta* was lowest. The ranking of the other species (*D. simulans, D. teissieri, D. mauritania,* and *D. yakuba*) varied depending upon sex of the flies. In all species, females survived longer than males.

Parsons (1979) assessed survival and fertility for the cosmopolitan species *D. melanogaster* and *D. simulans* (three isofemale lines each from near Melbourne, Victoria, Australia) at 0% relative humidity and as close to 100% relative humidity as possible, at various temperatures. His data show interspecific differences favoring *D. melanogaster*, strongly in some cases. At temperatures of 30–34°C, adult *D. melanogaster* and *D. simulans* survive short periods of time (>6 hours and <24 hours) at 100% relative humidity and are highly fertile, as compared with 0% RH. Results were consistent with geographical and seasonal patterns of distribution in the wild. Parsons concluded that *D. melanogaster* tended to replace *D. simulans* when temperatures, and more especially temperature fluctuations, became extreme.

Levine *et al.* (1986) used three populations of *D. pseudoobscura* (12 isofemale lines each) and a laboratory strain of *D. melanogaster* (Oregon-R) to test for desiccation resistance at 0% RH and 20°C for 18 and 24 hours. Desiccation resistance was greater in females than in the corresponding males, except for one population of *D. pseudoobscura*. The males and the females of the three populations differed significantly among themselves in their desiccation resistance. The females, but not the males, of *D. melanogaster* showed a consistently greater desiccation resistance than those of *D. pseudoobscura*. One of the three *D. pseudoobscura* populations had a distinctly greater percentage of survivors at both time periods than the other two populations. They concluded that the greater desiccation survival of one of the populations over the other two may reflect the fact the it contains a greater number of inversion heterozygotes and, therefore, is more heterotic than the other two populations. According to Levine *et al.*, linked complexes of genes through inversions reflects the evolutionary adaptation of this species to environmental stresses.

Rosa *et al.* (1989) used two sibling species, *D. melanogaster* and *D. simulans* collected at Laguna Verde, Veracruz, Mexico, to test for various traits including desiccation resistance. In their study, *D. melanogaster* consistently outperformed its sibling species *D. simulans* in desiccation resistance. They found that female flies were superior to males for desiccation survival in these two species (plus for their long-term laboratory control stock of Oregon-R). The recently collected strain of *D. melanogaster* and the Oregon-R laboratory strain (of *D. melanogaster*) were superior to *D. simulans* in all four sex and time (18 and 24 hours) combinations. The recently collected *D. melanogaster* were superior to the Oregon-R laboratory strain in all comparisons except for males at 24 hours which had no difference. They concluded that the decreased desiccation resistance of the Oregon-R flies kept under laboratory conditions for many generations is "understandable, as the food bottles are quite moist, thereby, eliminating much of the selection pressure for desiccation resistance that is exerted on flies living in nature."

Guzmán *et al.* (1989) conducted a long-term comparison of the sibling species *D. melanogaster* and *D. simulans* with respect to several traits including desiccation resistance for a 1987 collection from Laguna Verde. They found that *D. melanogaster* surpassed *D. simulans* in desiccation resistance. They found consistent results compared to a previous collection from a different site and season of the year demonstrating significantly greater desiccation survival of *D. melanogaster* compared with *D. simulans*. These two collections, with consistent results, were the same both spatially and seasonally, and were tested at different times in the laboratory. The desiccation data agree with the results obtained by others when species from widely separated populations were tested (McKenzie and Parsons, 1974).

Levine *et al.* (1989) reported the same as Rosa *et al.* (1989), plus the comparison of data obtained from two sites (I and II) over three collections, including two different seasons. They obtained similar results for desiccation resistance for a comparison between populations from the two sites at the same season of the year.

Rockwell *et al.* (1991) periodically collected the sibling species *D. melanogaster* and *D. simulans* from Laguna Verde, Mexico, over a three year period and tested them for desiccation resistance. They were interested in whether the traits were subject to seasonal, short-term, or long-term changes and, if so, whether these changes occurred in parallel fashion in both species. They found significant temporal changes for desiccation resistance between male and female flies of the same species: *D. simulans* after 4 hours, and *D. melanogaster* after both 8 and 12 hours. A comparison of sibling species by sex found *D. melanogaster* significantly more resistant to desiccation than *D. simulans* (except at 4 hours). They found no indication of seasonal variation in desiccation resistance of males or females from either species at any of the three time periods (4, 8, and 12 hours). They could not link desiccation resistance with relative frequency of the flies collected in the field, nor with the observed changes in relative species frequencies for these three collections. Also, they could not find any simple relationship between desiccation resistance and average monthly temperature or total monthly precipitation (change in desiccation resistance does not correspond to the change of the seasons).

daLage *et al.* (1989) used adults kept without food of a French *D. melanogaster* strain in humid and dry conditions to measure survival at nine different temperatures ranging from 5 to 31°C. An exponential increase of life duration resulted from a temperature decrease. This phenomenon did not extend over the whole temperature range. Below a threshold (11-14°C), the exponential increase was not observed and a reduction of life duration occurred at very low temperatures. This demonstrates some specific, deleterious effects of cold. Starved males always survived longer than starved females in the presence of water. Under desiccating conditions, females survived longer than males except at very low temperatures. Survival with water was always much longer than in dry conditions. The ratio of survival time in humid air over survival time in dry air, which is an accurate estimate of tolerance to desiccation, varied according to sex (it was higher in males) and to temperature (it was lowest at middle temperatures).

The selective history of an area may also play a role in determining the ability of an organism to resist extremes in climate conditions. Parsons (1981) found that ten species from non-tropical (mainly temperate zone habitats) showed much greater resistance to extreme zone habitats, and desiccation in the laboratory than nine tropical species.

Davidson (1988) used populations of *D. melanogaster* from climatically different habitats of a temperate zone (humid tropics and wet/dry tropics), and tested them for cold and desiccation tolerance and found them to vary. He concluded that the genetic heterogeneity present among and within these populations is a reflection of the prior selective history of the area, and, therefore, these populations show genetic differences in ability to withstand extremes of climate.

Parsons (1988) claims it is necessary to emphasize traits that show interactions with the habitat under conditions of environmental stress for an understanding of evolutionary change. Hoffmann and Parsons (1989a) reported on an integrated approach to environmental stress tolerance and life-history variation for desiccation tolerance in *Drosophila*. Metabolic energy (or the availability of it) provides a general measure of the environmental stress that can be tolerated by organisms. Increased tolerance to a range of environmental stresses should be associated with a reduction in metabolic rate in *Drosophila*. They present three predictions about genetic variation for stress tolerance: (1) increased stress tolerance will tend to be associated with decreased metabolic rate; (2) genetic correlations between tolerance to different environmental stresses will tend to be positive; (3) stress tolerance and life-history traits will tend to be genetically correlated.

Hoffmann and Parsons (1989a) tested these predictions by artificially selecting for increased desiccation tolerance in *D. melanogaster* using an 85% mortality level. They found a rapid response to selection (the mean realized heritability was 0.65). They found that the selection response was associated with a decreased rate of water loss, reduced activity, and a decrease in metabolic rate in agreement with prediction (1). Selected lines were relatively more tolerant of starvation and a toxic concentration of ethanol in agreement with prediction (2), and had lower fecundities in agreement with prediction (3). Hoffmann and Parsons found that *D. melanogaster* lines when selected for increased desiccation resistance have a lowered metabolic rate and behavioral activity levels, and show correlated responses for resistance to starvation and a toxic ethanol level.

Hoffmann and Parsons (1989b) studied the genetic basis of the selection response and extended the study of correlated responses to reactions to other stresses. They found that the response to selection was not sex-specific and involved both X-linked and autosomal genes acting additively. Activity differences were found to contribute little to differences in desiccation resistance between selected and control lines. Selected lines were found to have lower metabolic rates than controls in darkness when activity was inhibited. Adults from selected lines were found to show increased resistance to a heat shock, super (60)-gamma-radiation, and acute ethanol and acetic acid stress.

Overall environmental stress has a metabolic cost. An organism may increase its resistance by reducing its metabolic energy or expenditure. Parsons (1990) demonstrated this for a range of generalized stresses including: starvation, toxic levels of ethanol and acetic acid, irradiation, and high temperature. Parsons used *D. melanogaster* to morphologically illustrate that the norm of reaction can be upset by environmental (and genetic) changes which have costs at the developmental and metabolic levels. Lines of *Drosophila* selected for increased desiccation resistance were found to have decreased behavioral activity (they moved less), decreased metabolic rate, lower fecundity, and were resistant to an array of generalized stresses.

Increased resistance to combinations of environmental stresses should often be genetically correlated because of a reduction in metabolic energy expenditure due to a common genetic mechanism. Parsons (1990) concludes that an integrated approach to environmental stress is therefore emerging by considering "metabolic rate as the key trait, and using desiccation resistance as the environmental probe for magnifying genetic variability." Two other traits that are included in this overall environmental stress are vagility and body size, which are the subjects of the next two sections.

Vagility:

Vagility is the propensity of an organism to move from one place to another (Sakai *et al.*, 1958). This tendency to move may be driven by genetic factors, environmental factors, or the interaction of both. Of importance to evolutionary and behavioral biology is the examination of genetic and environmental factors influencing this propensity (especially from one geographic locality to another). Vagility is a behavior that is important in the natural setting of most animal species and, thus, is important to the study of evolutionary genetics of a behavior. These movements could lead to the establishment of new populations, or to maintain the genetic continuity of a population over a wider range. This review will investigate the studies of the interactive effects of genotype and environments on vagility, and the genotypic variation within natural populations for this trait.

Vagility includes searching for food, escaping from enemies, and finding a mate (besides migratory behavior in general). Flies feed when the humidity is high: soon after sunrise and before sunset. Since selection should favor individual flies confining their feeding activities to when the humidity is high, traveling into and out of these feeding areas would be important to maintain water balance (Arlian and Eckstrand, 1975). The interacting effects of genotypes and environments on the movements of individuals in a given population from one locality to another results from two processes: migration and dispersal. In Rockwell *et al.* (1978), the following definitions were given: Migration—the goal oriented movement of a fraction of the population; Dispersal—the movement of a fraction of a population as a result of the general (random) activity of its members; Emigration response behavior—the overall movement of organisms from their place of origin to a new location. Rockwell and Levine (1986a) used: Vagility—an individual's tendency to move.

It was found that adult *D*. persimilis and *D*. pseudoobscura flies may return to their area of origin when moved away (Taylor and Powell, 1983), and *D*. *subobscura* flies tend to return to the bait on which they were first collected (Atkinson and Miller, 1980).

Powell and Taylor (1979) studied habitat selection of populations of *D. persimilis* Dobzhansky and Epling (sibling species of *D. pseudoobscura*). Their study of chromosome-inversion types and protein alleles over several distinct ecological niches revealed that even over fairly short distances, inversion and gene frequencies differed from habitat to habitat. From their results, Powell and Taylor inferred that the microgeographic genetic variation of these populations may reflect adaptations to their respective habitats. They used a capture-release-recapture experiment using UV-fluorescent dust of different colors, and found that there was a distinct tendency for flies to return to the area, in which they were captured initially.

However, Coyne and coworkers (1987), studying how far flies can travel, found genetic uniformity of apparently isolated populations. They tested dispersal of three *Drosophila* species (*pseudoobscura*, *melanogaster*, and *simulans*) in the desert of Death Valley, California, by seeking flies in desert locations far from apparent breeding sites and by monitoring the movement of flies released at various distances from a large oasis. The striking genetic uniformity of apparently isolated *Drosophila* populations has led Coyne *et al.* to the hypothesis that they are connected by occasional long-distance migration. Coyne *et al.* caught flies in many remote desert locations as far as 26 km from the nearest likely breeding site in the spring. Flies are not found at these sites in the middle of summer, and, therefore, must travel long distances to get there.

Coyne and Milstead (1987) released one million marked *D. melanogaster* individuals to measure the amount of long-distance movement of genes in a temperate area. To model natural movement as realistically as possible, the flies were marked by heterozygosis for two linked recessive alleles, released as pupae and not as adults, and release over a two month period in an isolated Maryland orchard. They found marked flies in appreciable numbers up to 6 km from the release site three months later (with some released alleles traveling as far as 10 km). They suggested that *D. melanogaster*, in northern North America, consists of demes that are regularly connected by migration, but the adults die off in winter.

Studies of vagility in natural populations of *Drosophila* are limited. Several studies addressed the norm of reaction by investigating the interactive effects of genotype and environments on vagility (Tantawy *et al.*, 1975; Rockwell *et al.*, 1978; Rockwell, 1979; Mikasa and Narise, 1983). Other studies estimated the level of genotypic variation within natural populations.

Narise (1968) studied the relationship of vagility to fitness using wild type and mutant laboratory strains of *D. melanogaster*. Separate groups of hybrids between wild type and vestigial wing flies were placed in either population cages or in double-ringed vagility apparatus. The frequency of vestigial flies deceased generation after generation in both populations, with significant differences in the pattern of selection against the vestigial and its final frequency. Although selection was against the vestigial trait, the ability of vestigial flies to move about in a partitioned environment of the vagility apparatus appeared to have permitted the retention of the vestigial allele in the population.

Narise (1974) examined the relationship between vagility and fitness of one wild type and five mutant strains of *D. melanogaster* using the Sakai single-ringed system. Several components of fitness—fecundity, viability, and longevity—were examined. Fecundity and viability were found to be negatively related to vagility, while longevity was positively related (all not significantly so). Narise concluded that there tends to be a greater vagility with lower fitness.

The effects of chemical communication on vagility was investigated by Narise and Narise (1990). They used six homozygous strains of *D. melanogaster*, examined whether or not a substance(s) secreted by one strain affected the emigration behavior of another strain. The experiment involved the flies of one strain in a central tube being discarded and those of another introduced. They found that in some combinations, emigration behavior of the latter strain was influenced by a substance(s) secreted by the former strain.

Sensory cues perceived by adult *Drosophila* can affect their dispersal behavior. The distribution and abundance of the sexes in populations of some *Drosophila* species could be affected by non-sexual communication (Parsons and Hoffmann, 1985). Parsons and Hoffmann found that *D. melanogaster* and *D. simulans* males are attracted to trace odors from homotypic females. A possible explanation is an inhibitory pheromone associated with *D. melanogaster* males and mated females (Scott, 1986).

Pino *et al.* (1991) compared dispersal of virgin and non-virgin *D. melanogaster* and *D. simulans* in the presence and absence of conspecifics. They chose dispersal because this behavior is likely to have consequences for the distribution of flies in feeding strategies, selection of oviposition sites, and utilization of space. Pino *et al.* found that adults of *D. melanogaster* and *D. simulans* transmit and receive signals at some distance from their conspecifics. Some flies may increase their dispersal in response to some substances such as pheromones. Pino *et al.* concluded that non-sexual signals between conspecifics at some distant could have consequences for the distribution and abundance of *Drosophila* species by serving as social indicators in habitat selection.

Vagility has been studied in the laboratory by a number of researchers using a system devised by Sakai and coworkers (1958). This system consists of four interconnected vials, one central and three peripheral through which individual flies can move.

Several researchers (Sakai *et al.*, 1958; Mikasa and Narise 1979, 1983) compared the vagility of *D. melanogaster* geographic strains. Sakai *et al.* (1958) compared the vagility of six geographic strains of *D. melanogaster* and found differences among them. In addition, Narise (1962) was able to select for increased vagility of recently captured flies and found a marked response to selection. Mikasa and Narise (1979, 1983) compared *D. melanogaster* collected from small islands and large adjacent land masses (mainland) Japan, and found that the amount of movement exhibited by flies from the former was generally less than that exhibited by flies from the latter.

While other studies estimated the level of genotypic variation within natural populations, they did not include either spatial or temporal dimensions. Rockwell (1979) studied the effects of both light and height of arms of connecting tubes on the movement of *D. busckii* collected from Ontario, Canada, and Redding, Connecticut. In both experiments, there was no effect of light on vagility but there was an effect of height of connecting tube arms. He showed that the tube height by lighting condition interaction was highly significant. The interdependence of tube height and lighting condition on the emigration response behavior of *D. melanogaster* and *D. busckii* is different. For *D. melanogaster* they are not independent, and for *D. busckii*, tube height modulated the emigration response showing a greater emigration response in the low tubes with no apparent effect of lighting condition. Rockwell concluded that the overall norm of reaction for *D. busckii* appeared to be narrower and less complex than that of *D. melanogaster* for this behavior. He believes that these results may show that the reduced plasticity of this behavior in *D. busckii* may be related to the reduced heterozygosity of this species.

Rockwell and Levine (1986b) tested *D. busckii* (8 isofemale lines) for emigration response behavior using a modification of the Sakai system analyzing two environmental variables: height and light. They found significant variation in the behavior of the isofemale lines in all four environmental configurations. They suggested that this demonstrates "the high degree of genotypic variation for emigration response behavior present in this natural population of *D. busckii*." There was no main effect of light, but there was an effect of height on the emigration response of the isofemale lines. The isofemale lines differed in the overall pattern with which light and height affected their behaviors. They concluded that in this population of *D. busckii*, "there is a varied and complex assemblage of genomes affecting emigration response behavior." "The genotypic variation in this control, particularly when coupled with genotypic variation in the individual's basic tendency to move, affords a population evolutionary flexibility when confronting altered and even novel environments."

Rockwell *et al.* (1983, 1986a) found that vagility was strongly correlated with the populations' level of chromosomal polymorphism for *D. pseudoobscura*. They used twelve isofemale lines of three Mexican *D. pseudoobscura* populations and tested them for vagility (single-ringed/high tubes) in the Sakai system to investigate the behavioral characteristics of the flies of these populations. Flies from the most chromosomally polymorphic population had both a significantly lower vagility score and a higher level of expressed genotypic variation for vagility than the flies from the lesser chromosomally polymorphic populations.

Comparing these results to studies of later collections, Rockwell and Levine (1986a) and Rosa *et al.* (1984) found that in the earlier *D. pseudoobscura* sample that the difference for the highly polymorphic population, which possessed a much lower mean vagility score than either of the lesser polymorphic populations, had disappeared in a later sample. The mean response of all three populations had decreased during the interim—markedly so for one. These findings follow a hypothesis of selection for less population migration during the favorable season of the year and the reverse during the harsh season, with increased amounts of genetic variability of a population during the favorable season. They concluded that only future temporal studies could determine whether this temporal change is a gradual time related shift or a seasonal effect, and whether the change is cyclic or stochastic.

Using collections from two different sites from Laguna Verde, Veracruz, Mexico, Levine *et al.* (1989) tested vagility for *D. melanogaster* and *D. simulans*. Flies from one of the sites showed only a significant difference in vagility between the species at 24 hours in one collection. In comparing seasonal differences within a site, vagility was found to be significantly different (significant seasonal difference).

Guzmán *et al.* (1989) conducted a long-term comparison of the sibling species *D. melanogaster* and *D. simulans* with respect to several traits including vagility for a 1987 collection from Laguna Verde, Veracruz, Mexico. They found contrasting results compared to a previous collection from a different site and season of the year: greater vagility of *D. melanogaster* at both time periods versus no difference between species (1987 collection). These two collections, with contrasting results, differed both spatially and seasonally, and were tested at different times in the laboratory. They excluded any possible changes in the laboratory as a cause of the observed differences between collections based on the results of an Oregon-R (*D. melanogaster*) laboratory control stock. They presented two other possibilities: that the differences between collections reflect genetic differences, or the observed results modified their respective populations in different ways. The final conclusion of these differences will depend on further studies of these populations.

Rosa *et al.* (1989) also collected the two sibling species, *D. melanogaster* and *D. simulans* at Laguna Verde, Veracruz, Mexico. They were tested for various traits including vagility. *D. melanogaster* were significantly more vagile than was *D. simulans*. There was no statistically significant difference in vagility between the collected *D. melanogaster* and the Oregon-R (*D. melanogaster*) laboratory (control) strain of that species.

Extending these studies to include more collections over more seasons, Olvera *et al.* (1991) periodically collected the sibling species *D. melanogaster* and *D. simulans* from Laguna Verde, Veracruz, Mexico, over a three year period and tested them for vagility in a double-ringed Sakai system. They found no indication of seasonal variation in dispersal activity of either species for either the 4 hour or the 24 hour time period. Both species showed significantly higher dispersal scores at 24 hours than at 4 hours, and the difference in dispersal scores between the two time periods was identical for both species. *D. melanogaster* exhibited significantly higher dispersal scores than *D. simulans* at 4 hours in three of the six collections and in an overlapping group of three collections at 24 hours.

Also, Olvera *et al.* (1991) could not link changes in dispersal activity to increasing relative frequency of *D. melanogaster* in the last three collections, nor to observed changes in relative species frequencies with the significant dispersal score differences within each of the species. There was no change in dispersal scores due to seasonal change.

Since vagility—the tendency to move—is an important component of dispersal, emigration response behavior, and migration, it is an important factor in the evolutionary process because differences in this tendency could be adaptive under changing ecological conditions (e.g., alternating seasons). Genotypic variation in the genetic control of vagility has been shown by several investigators, and may give a population the evolutionary flexibility to adjust to or escape from a changing environment. Also, vagility may be related to fitness, as found by Narise (1974), a greater vagility tends to be accompanied by a lower fitness. Other fitness traits may be correlated with vagility such as body size—which is the subject of the next section.

Body Size:

One of an organism's most obvious features—its size—is an attribute that is limited by physical constraints. For flying insects, maximum size may not be constrained by aerodynamics, but by physiological factors. Even with the physical or physiological constraints, body size can still vary enormously. Ecological factors may play an important role in this variation. The evolution of body size can be understood either within the narrow sense of genetic change or in the broader sense of phenotypic or genotypic change, but does not occur in isolation from other life history phenomena (Roff, 1986).

Body size can be used in a simple demographic model to address life historical and ecological constraints (Roff, 1986). Two parameters of Darwinian fitness, survival and fecundity, can generally be related within a species to body size (Roff, 1981, 1986). For invertebrates, fecundity generally increases with body size (Roff, 1981). Since body size is strongly integrated with life history traits, it represents an index by which one may judge how well these traits can be identified and defined (Roff, 1986).

Characters correlated with a life history trait, such as body size, almost certainly are subject to natural selection (Prout and Barker, 1989), and the response to such selection depends on heritability. Since flies of the genus *Drosophila* exemplifies the colonizing lifestyle, Roff (1986) predicts that natural selection will favor life history characteristics in *Drosophila* that maximize the instantaneous rate of increase.

According to Roff (1986), there are tradeoffs among life history characters related to body size, therefore, there should be some optimum body size in *Drosophila*. Large flies have a higher fecundity, but fewer of them survive to reproduce and, thus, do not fully realize this potential. An increase in body size increases fecundity but does not change the shape of the fecundity curve (Roff, 1986). Luckinbill *et al.* (1989) examined three likely traits for their possible connection with increased life span in strains of *D. melanogaster* selected for longevity. They found that variation in body size does not appear significantly to contribute to longevity.

Thorax length (Robertson and Reeve, 1952) and wing length (Curtsinger and Laurie-Ahlberg, 1981) have been used as indications of body size. Robertson and Reeve (1952) investigated the effects of selection on thorax and wing length in *Drosophila melanogaster*. They demonstrated a strong genetic and environmental correlation between wing length and thorax length. Since wing length correlates with thorax length, wing length can also be used as a measure of body size. Wing characters may be chosen instead of thorax length for study because the wing can be treated as a two-dimensional object and its various dimensions measured with relative ease and high accuracy (Curtsinger and Laurie-Ahlberg, 1981). In addition to their use in flight, wings function in the mating behavior of males, another possible source of selective differences (Curtsinger and Laurie-Ahlberg, 1981).

Wing length has been shown to respond to selection. In 1953, Reeve and Robertson analyzed a strain of *D. melanogaster* selected for long wings and reported that wing morphology responded readily to artificial selection. But Prout (1958) investigating differences in genetic variance between wild and laboratory populations of *Drosophila* found a heritability (under natural conditions as opposed to an experimental environment) for body size equal to zero in a citrus grove for *D. melanogaster*. Coyne and Beecham (1987) studied the heritability under natural conditions of body size in *D. melanogaster* and found greater than zero heritability.

There is direct evidence that wing morphology is subjected to selection in the laboratory. Barron (1982) reported directional change in wing shape over many generations in laboratory populations of *D. melanogaster* and some related species. He argued that natural selection modifies wing morphology to cope with the novel aerodynamic demands of flight in a laboratory environment. He found that *D. melanogaster* wing morphology evolves as the lines adapted to laboratory culture. Barron reasoned that the longer a line is exposed to the laboratory environment, the more likely it is to have undergone selection on wing morphology, reducing additive genetic variance for the character. There has been rather intense natural selection operating on the wing characters in the laboratory populations (Curtsinger, 1986). There appears to be very little additive genetic variance for the wing morphological characters in the outbred lines, while there was a substantial maternal effect (Curtsinger, 1986).

Lints and Bourgois (1987) used Oregon-R, a wild type laboratory stock of *D. melanogaster*, which they divided into three subpopulations and submitted to different environmental temperatures. Over six years, duration of development, thorax size, and male wet weight were measured several times in the three subpopulations. After 36 weeks, they observed genetic divergence among the subpopulations. They discussed different hypotheses which may explain the origin of the genetic variability present in subpopulations derived from a laboratory stock maintained in a constant environment during more than 15 years.

Budnik *et al.* (1988) found that wing length and width increased with elevation above sea level in *D. flavopilosa* for males. Females however, exhibited a different type of variation: the larger and wider wings were found in the populations at intermediate elevation. Their results support the idea that males and females react in a different way to selective pressures affecting wing development.

According to Roff (1986), the most reasonable explanation for the low heritability estimates is that the characters have been subjected to natural selection which has reduced the additive genetic variance. Prout's data do not allow determination of the exact mode of selection affecting wing morphology. It also is possible that wing morphology changes because of selection on other characters with which it is correlated, such as body size.

Curtsinger (1986) tested quantitative genetic predictions regarding variance components and heritabilities. He undertook an experimental check on the theoretical expectations of quantitative genetics applied to certain specially constructed stocks of *D. melanogaster*. He used variation of two wing morphology characters by studying 11 X-chromosome substitution lines and three outbred lines of *D. melanogaster* and found between-lines components of phenotypic variance were statistically significant—the significant within-line component was attributable to a maternal effect. There was very little or no genetic variation within lines, and that maternal effects accounted for much of the variation observed between pairs within lines. Curtsinger found unusually low broad-sense heritabilities in the inbred lines, and unusually low narrow-sense heritabilities in the outbred lines. He concluded that the most likely explanation for the low heritability estimates was due to operation of natural selection on wing or correlated characters as stocks adapt to the laboratory environment.

Starmer and Wolf (1989) examined influences on wing and body size in 11 species (12 strains) of *Drosophila.* Six measures of wing length and width were closely correlated with wing area and suggested little variation in wing shape among the species. Among ten of the species, wing loading (an important factor in flight costs and maneuverability) increased as body mass increased at a rate consistent with expectations from allometric scaling of wing area and body mass to body length. Intraspecific variation in wing loading showed similar relationships to body mass. Density and temperature during larval development influenced wing loading through general allometric relations of body size and wing area. Temperature during the pupal stage, but not during wing hardening after eclosion, influenced wing area independent of body size. Wing area increased as growth temperature decreased. Individuals reared at cooler temperatures, thus, compensated for a potential allometric increase in wing loading by differentially enlarging the wing area during pupal development.

Variation in other traits have also been found. Such variation was demonstrated for fecundity (Tantawy and El-Helw, 1966), and body size and development time (Tantawy and El-Helw, 1970) in natural populations of *Drosophila.* Male longevity and size were found to be correlated in the laboratory (Partridge and Farquhar, 1983). Curtsinger and Laurie-Ahlberg (1981) observed wing morphology to exhibit moderate to high levels of genetic variance in *Drosophila* populations.

Budnik *et al.* (1988) found sexual dimorphism to be significantly different between the studied populations. Also, larger size may have an advantage for mating male *Drosophila*. Ewing (1961, 1964) investigated the relationship of body size and courtship behavior and the influence of wing area on courtship of *D. melanogaster*. He found that body size has a major influence on male mating success in *D. melanogaster* in the laboratory. In *D. melanogaster*, male size has been shown to correlate with short-term measures of mating success in the field and laboratory. Partridge and Farquhar (1981, 1983) found that large males are more likely to win aggressive encounters. A male's mating success and his access to females can increase if he is successful in defending patches of food against other males (Hoffmann and Cacoyianni, 1988). Partridge and Farquhar (1983) found that in *D. melanogaster* body size has been a major influence on male mating success in *D. melanogaster*. In the laboratory, body size has a major influence on male mating success in *D. melanogaster*. Partridge and Farquhar (1983) found that in *D. melanogaster*, male size has been shown to correlate with lifetime measures in the laboratory.

Partridge *et al.* (1987a) found that larger *D. melanogaster* males delivered more courtship to virgin females, produced more courtship song, and sang more loudly than smaller males. Partridge *et al.* used the approach of non-random mating in the absence of any opportunity for male rivalry to investigate the roles of male and female behaviors producing the higher mating success of large *D. melanogaster* males. Larger *D. melanogaster* males also moved around more when not courting. Females seemed not to discriminate between males of different sizes, but that scramble competition between males to deliver courtship is important in determining mating success.

Partridge *et al.* (1987b) used *D. melanogaster* and showed that male size was correlated with short-term measures of mating success in the field and laboratory. Wild mating male *D. melanogaster* and *D. pseudoobscura* were larger than randomly sampled males. Larger males were found to have a mating advantage with virgin and inseminated females and delivered more courtship. Females do not seem to discriminate between males of different sizes, but scramble competition between males during courtship is important in determining mating success under field conditions too.

Steele and Partridge (1988) found that large male *D. subobscura* can carry bigger drops of regurgitated food during courtship and, therefore, they have greater courtship success with starved females than do small males. But in opposition to the above references, if males are prevented from producing a drop, the small males have greater courtship success than the large males, suggesting that they perform some other aspect of courtship better than large males. In their study, small males were better than large ones at tracking the female during the courtship dance. Males of different sizes run at the same top speed during the dance, but the larger males lagged further behind the female presumably because they take longer to accelerate to or decelerate from any given speed. This difference may be explained by Partridge *et al.*'s (1987a) finding that fighting between males may be important in *D. pseudoobscura*, but not in *D. melanogaster*.

Hoffmann (1989, 1991) collected *D. melanogaster* males from two field sites and tested them for territorial success in the laboratory. He also found that body size variation (measured as weight) in the wild has a large influence on territorial success. However, this variation was not transmitted to the next generation (unlike variation in territorial success). Hoffmann concluded that body size may not be an important component of heritable variation for territorial success for these flies. Therefore, heritable variation for territorial success in male flies for this population of *D. melanogaster* does not seem to involve genes influencing body size. This absence of heritable variation for body size with wild flies may reflect gene-by-environment interactions or differences in the size of the additive genetic variances and environmental variances in the wild and the laboratory.

Others have investigated the heritability of body size when subjected to the environmental variation experienced in nature (as opposed to an experimental environment). Prout and Barker (1989) reported results of an investigation of the heritability of body size in *D. buzzatii* when subject to this environmental variation. They took advantage of the well defined ecology of the cactophilic *D. buzzatii* to analyze the heritability of body size within and between subdivisions of a single natural population. They concluded that for the laboratory environment, h super(2) = 0.3770 ± 0.0203 and for the natural environment h super(2) = 0.0936 ± 0.0087 within rots and h super(2)= 0.0595 ± 0.0123 for a population drawn randomly from different rots.

Implicit in the following discussion of heritabilities and genetic variance of body size is the assumption that *D. buzzatii* is like *D. melanogaster* in that maternal effects and dominance variance are of little importance (Robertson and Reeve 1955; Robertson 1987).

The principal objective of Prout and Barker's (1989) experiments was to determine the heritability of body size in the natural environment. This was also the objective of Coyne and Beecham (1987) with *D. melanogaster*. Prout and Barker's results agree with theirs that heritability in the wild is less than in the laboratory. One of the objectives of both experiments, however, was to determine the amount by which heritability is reduced. The decrement in the case of Prout and Barker (1989) is about the same as that of Coyne and Beecham (1987). The absolute values of Prout and Barker's wild and laboratory heritabilities (across rots) were less than Coyne and Beecham's. But this kind of difference would not be unexpected when comparing different species which live in different habitats. For the case of heritability of body size in the natural environment, there may be net stabilizing selection on body size in *Drosophila*, which explains why flies are not larger, but does not explain the existence of the genetic variation.

Body size (which can be measured through wing length) is an important fitness trait. The evolution of body size occurs in conjunction with other life history traits. Ecological factors may play a role in the enormous variation that may occur in body size. According to Prout and Barker (1989), selection depends on heritability and heritability may depend on ecological factors. Other characters may be correlated with body size, such as desiccation resistance and vagility, and would be subjected to natural selection.

MATERIALS AND METHODS

Stocks and Maintenance:

The stocks of *D. melanogaster* and *D. simulans* used in this project were isofemale lines of naturally sympatric populations. The Mexican scientists randomly collected the flies with baits of fermenting pineapple. After collection, vials with flies were brought to a microscope set up in the field, and the sexes were separated. Species can be identified either directly through an examination of the males or indirectly through an examination of the sons of individual females. Thus, the experimental flies of *D. melanogaster* and *D. simulans* flies came from isofemale lines, each consisting of the descendants of an individual female that had mated in the wild. Ten such lines from each species were utilized in each of the three experiments, plus the Ives Oregon-R laboratory stock of *D. melanogaster*. This was used with each of the four collections.

The isofemale lines of the two species (and the Oregon-R laboratory stock) were maintained until testing, within three generations after capture, in half-pint milk bottles on blue-colored Carolina Instant *Drosophila* Medium (from the Carolina Biological Supply Company) and yeast at 20°C under constant illumination. Maintenance under constant illumination randomizes the photoperiods within and between the isofemale lines and, therefore, behavioral analyses can be carried out with minimum bias for a longer part of the day (Rockwell, 1971). The relative positions of all of the replicate cultures in the incubator were randomized.

All collections of flies were performed under low amounts of humidified carbon dioxide as the anesthesia. Recovery from humidified carbon dioxide requires approximately two minutes.

Desiccation Resistance:

Twenty-five males and twenty-five females (4–7 days old) were collected from culture bottles for each isofemale line for *D. melanogaster* and *D. simulans* and the Oregon-R laboratory stock and were placed in 8 dram vials with food (white Instant *Drosophila* Medium / no yeast), which were then stored in an incubator (20°C, constant light) until the next day. All anesthetizations were performed with humidified carbon dioxide.

The next day, 20 flies (males and females separately) were placed in an empty 4 dram vial with a cap covered with netting. These flies were left undisturbed for approximately one hour in the incubator.

The vials containing these flies were then placed into desiccators containing fresh indicating Drierite, and were placed in a "walk-in" incubator (20°C, constant light). The experiments were begun at 7:00 a.m. and readings of the number of live flies were taken at 4, 8, 12, and 24 hours. After the 24 hour reading, the remaining live flies (if any) were anesthetized and a "dead count" on all flies in the vial was performed to check whether the density was 20. (Densities between 18 and 22 are acceptable.) The number of live flies at each time for each isofemale line and sex is divided by the density to obtain a desiccation proportion (desiccation score).

Six replicates of males and females were run for every isofemale line of both species and for the Oregon-R laboratory stock yielding results based on 2520 male and 2520 female flies (assuming a density of 20 flies each time); or 20,160 flies for all four collections.

Vagility:

Fifty-five adult male flies (4–7 days old) were collected from culture bottles for each isofemale line for *D. melanogaster* and *D. simulans*, and Oregon-R. Each set of 55 were placed together in 16 dram food (white Instant *Drosophila* Medium / no yeast) vials and stored at 20°C for approximately three hours. All runs were started at 10 a.m. Humidified carbon dioxide was used as the anesthesia for the procedure.

Prior to the beginning of the experiment, ten vagility vials were connected with transparent tape to form an adult double-ringed vagility test system (see Figure 2). The unused arms of the connecting tubes of the three peripheral vials were plugged with corks and the tops of the vials were closed off with plugs. White Carolina Instant *Drosophila* Medium, with no yeast, was used as the food source in the vials. Each assembled system was placed in an open cardboard box which was painted white inside to reflect diffuse light. The boxes containing the adult double-ringed vagility test system was placed in a 20°C "walk-in" incubator, 61 cm beneath a fluorescent light fixture consisting of two 40 watt bulbs. Up to six double-ringed vagility boxes can be used at the same time. Positions of each isofemale line in its box was randomized during the three replicates.

At the start of the experiment, a given set of 50 males (of the 55 collected flies) was aspirated into the central vial of the system. (Collecting 55 flies, instead of 50, guarantees the correct number in case of some deaths.) At 4 and 24 hours (a relatively short and long time period), the number of flies in each vial of the adult double-ringed vagility system was counted (minus that of the center vial). Sums of the number of flies in each ring (inner and outer rings) were calculated. The total number of flies in the three vials of the inner-ring and the six vials of the outer-ring are used as the measure of vagility, and the number of flies in the rings can range from 0 to 50.

After the 24 hour reading, the flies in the adult double-ringed vagility system were anesthetized and a "dead count" of the number of flies in each vial (including the center vial) was made. This tells the density of flies (a check on whether 50 were actually introduced into the system). Densities between 46 and 54 are acceptable.

With this density number, a vagility score was calculated for the 4 and 24 hour readings:

Vagility Score =

$$\boxed{\frac{(1)\ (\text{\# of flies in the inner ring}) + (2)\ (\text{\# of flies in the outer ring})}{(2)\ (\text{Density})}}$$

Each run in the experimental system was replicated three times, using a new group of 50 flies each time which was obtained from a different culture bottle. This required 30 runs for each species (10 isofemale lines each), plus 3 runs for Oregon-R, for each collection. Thus, tests were made on 3150 flies (assuming 50 each time) in adult double-ringed vagility for each collection (totaling: 12,600 flies for all four collections).

Body Size:

Random replicate samples of males and females of all of the isofemale lines of *D. melanogaster* and *D. simulans* and the Oregon-R laboratory stock were collected and preserved in 70% ethanol. These flies were of the same generation and age as those tested for desiccation resistance and vagility. The wings were removed, mounted, and measured (separately for males and females) using the procedure below.

All of the storage ethanol (70%) was decanted and the flies were immersed in 95% ethanol. For a given isofemale line, five male and five female flies were chosen on the basis of good wing condition. (Occasionally, very small flies were encountered and avoided.) The right wing was removed by clipping at the base of the wing. The five wings were positioned on the slide next to each other in the same direction. The slide was moistened with the 95% ethanol to enable the wings to be moved about easily. The 95% ethanol was allowed to evaporate. Using the tip of a small applicator, the slide was moistened with xylene in the vicinity of the wings. By touching the edge of the wings with the applicator, xylene flowed onto the surface of the wing helping it to adhere to the slide. The excess xylene was allowed to evaporate.

A solution of mounting medium diluted with xylene (5%–10% by volume depending on the ambient temperature and desired flow characteristics) was applied to the coverslip. The coverslip was placed on the wings. Occasionally, it was necessary to apply a small amount of xylene to the edge of the coverslip to help draw out the mounting medium to fully encapsulate the mount.

Size was measured as wing length of the III longitudinal (L3) vein (in mm), taken from the intersection of the anterior cross vein and L3 to the intersection of L3 with the distal wing margin (Partridge *et al.*, 1987a) (see Figure 3). A stereoscopic microscope equipped with a digital micrometer was used for the measurement.

Each slide has five mounted wings for a particular species, isofemale line, and sex. Each of these wings on a slide represent one replicate for a total of five replicates. Thus, results are based on 105 male and 105 female flies; or 840 flies for all four collections.

Thorax length is the other commonly used measure of size (Robertson and Reeve, 1952). Strong genetic and environmental correlation between wing length and thorax length was demonstrated by Robertson and Reeve (1952); but wing length is a more convenient measure when the flies can be killed (Partridge *et al.*, 1987a).

Statistical Analyses of Data:

In the experiments (for all collections), independent replicate trials were performed on all isofemale lines of each species' population in a replicate blocks fashion (Winer, 1971). In this design, a given replicate of all lines is completed before the next replicate of any line is begun. The individuals for each replicate of a given isofemale line are drawn randomly from a different culture bottle of the line. This procedure maximizes the likelihood that any culture bottle differences between lines will be represented within lines and, thus, minimizes the potential effect of common environmental variation on similarities within isofemale lines.

The mean desiccation resistance, vagility, and body size measurements were calculated for each isofemale line. In addition, grand means, calculated over the isofemale lines, were calculated for each species. The effect of seasonal differences, as well as the level of isofemale line differentiation, for desiccation resistance, vagility, and body size was assessed separately with a cross-nested analysis of variance. In this analysis, isofemale lines have a hierarchical relationship to the factorially related variables. This analysis also indicates any interdependence between the environmental variables for each of the traits studied. Specific comparisons of means between annual and seasonal samples were made.

To test whether the factors of year, season, species, and sex are independent of each other and what differences exists in the data for these factors, univariate factorial analyses were performed. Three-, two-, and one-way analyses of variance were performed to examine for differences in the data for these traits. All traits were considered simultaneously with multivariate analyses and correlation to test whether these traits are analogously (functionally) related.

Since the isofemale lines were raised and tested in the same environment and since flies were drawn from different replicate cultures, the component of variance due to isofemale lines is a measure of the "expressed genotypic variance" for each of the measures of a given sample (Rockwell *et al.*, 1975a,b; Rockwell, 1980). Since the lines were randomly collected from nature and exposed to a minimal amount of laboratory selection, the expressed genotypic variance reflects the broad genotypic structure of these measures for the natural populations of these flies. The levels of isofemale line differentiation (levels of genetic variance) were evaluated for each combination of year and season. These were estimated as added components of variance from a series of one-way analyses of variance. Comparisons between pairs of estimates were made with a Monte Carlo procedure.

Estimates (Sokal and Rohlf, 1981) and confidence intervals (Boardman, 1974) of the added component of variance can be calculated, but the distribution of the ratio of these estimates is unknown and cannot be assumed to follow an F distribution. A C-language computer program was prepared to examine the distribution of this ratio, for specified values of the added and error variances and number of treatments (lines within a population) and replicates, by running a large number of simulations (100,000) of Monte Carlo trials. In each run, observations for the two samples were generated via a normal random variable function, the two analyses of variance were carried out, the two added components of variance estimated, and the ratio was formed. These simulations allows a conclusion on the probability of this ratio exceeding a stated value under these specified conditions. (See Rockwell *et al.*, 1975a,b, and Rockwell, 1980.)

RESULTS

The mean responses are important measures of the phenotypes in the current study. Desiccation proportions, vagility scores, and body size measurements were averaged for each species (10 isofemale lines) in each collection, and for desiccation resistance and vagility for each time period. Males and females were treated separately for desiccation resistance and body size. Vagility examined males only.

Isofemale line means and their associated standard errors of desiccation resistance proportions for *Drosophila melanogaster* and *D. simulans* for males and females separately are given in Table 1 for all four time readings (4, 8, 12, and 24 hours). They are depicted in Figures 4–7 by collection for males and females for each time reading.

Isofemale line means and their associated standard errors of vagility scores for *Drosophila melanogaster* and *D. simulans* for males (only) are given in Table 2 for both time readings (4 and 24 hours) and depicted in Figures 8 and 9 by collection for each time reading.

Isofemale line means and their associated standard errors of body size measurements for *Drosophila melanogaster* and *D. simulans* for males and females separately are given in Table 3 and depicted in Figure 10 by collection for males and females.

Oregon-R (of *D. melanogaster*) was used to determine if laboratory conditions had changed and whether an "Oregon-R correction" was needed for the results for *D. melanogaster* and *D. simulans*. To determine this, two-factor analyses of variance for desiccation resistance, vagility, and body size were performed.

Oregon-R showed several significant ($p \leq 0.05$) main effects and interactions for year and month (season) for desiccation resistance. Thus, an Oregon-R correction was used for desiccation resistance, and the means of Oregon-R desiccation resistance scores at each time reading, for males and females separately, were subtracted from the scores for *D. melanogaster* and *D. simulans* before any further analysis. There were no significant effects ($p \geq 0.05$) for Oregon-R for vagility and, therefore, no correction was necessary. The vagility scores were angularly transformed to stabilize the sample variances before further analysis. There were also several significant main effects and interactions for year and month (season) for body size and, thus, an Oregon-R correction was used for this trait also.

To examine the effects of year, month, and species, and sex for desiccation resistance and body size on the means of the three traits, analyses of variance were performed. Initially, joint effects were examined to evaluate whether the factors were independent. Because the nature of any interactions and the pattern of effects at all single factor levels were of primary interest, a hierarchical set of reduced order ANOVA's was used.

For desiccation resistance and body size, analysis began with four-factor ANOVA and for vagility with three-factor ANOVA. All possible 3-, 2-, and 1-factor ANOVA's were then performed using each factor or factors in turn as a conditioning variable. The basic plan is illustrated in Figure 11. As indicated in the figure, not all of the possible analyses had to be performed since many of the "branch points" are redundant. In the tabular summary of these analyses, only non-redundant ones are included. Each trait—desiccation resistance, vagility, and body size—is analyzed separately, and for desiccation resistance and vagility, each time reading is also treated separately, as are males and females.

When discussing interaction terms, the following terminology was adopted. A significant interaction term indicates that the effects of the factors involved are *interdependent.* Non-significant interaction terms imply that these effects are *independent* (not dependent).

The four-factor ANOVA for four hour desiccation resistance is summarized in Table 4. There is a marginally significant fourth-order interaction ($p=0.06$), implying that the joint effects of the four factors are independent. There are, however, some significant (dependent) third-order interactions, that are more appropriately addressed in the following lower order ANOVA tables.

The three-factor ANOVA's are summarized in Table 5. The third-order terms are significant only in the analyses conditioned for month=7 and for sex=male. In those two cases, the effects of the other three factors are interdependent. The factors in the other analyses are all independent.

The two-factor ANOVA's are summarized in Table 6. The joint effects of year and month are interdependent for *D. simulans* (males and females), but independent for *D. melanogaster* (both sexes). The joint effects of month and species are interdependent for year=2 (males and females), but independent for year=1 (both sexes). The joint effects of species and sex are interdependent for year=1 (month=7 and month=1), but independent for year=2 (both months). There are no significant joint effects for month and sex, and these factors are independent. The joint effects of year and species and of year and sex show no consistent pattern of significance.

The patterns of first-order effects are depicted for each factor in Figure 4 and the analyses summarized in Table 7. There is a significant year effect in month=7 for both sexes for *D. simulans* (year=2 > year=1), but not for *D. melanogaster*. There is no pattern of significant month effects. The effect of species is significant across three of the four combinations for year=1. All except females in year=1 and month=7 are significant (*D. melanogaster* > *D. simulans*). Also, both sexes show a significant species effect for year=2 and month=1 (*D. melanogaster* > *D. simulans*). There is a significant sex effect in year=1 for both month=7 and month=1 for *D. simulans* (females > males). There is no other pattern of significant sex effects.

The four-factor ANOVA for eight hour desiccation resistance is summarized in Table 4. There is a significant fourth-order interaction, implying that the joint effects of the four factors are interdependent.

The three-factor ANOVA's are summarized in Table 8. The third-order terms are significant in the analyses conditioned for year=1, month=1, species=*D. simulans*, and for sex=female. In these cases, the effects of the other three factors are interdependent. The factors in the other analyses are all independent.

The two-factor ANOVA's are summarized in Table 9. The joint effects of species and sex are interdependent across all combinations of the conditioning variables. The joint effects of month and species are also interdependent for three of the four combinations. Only in females for year=1 is the interaction not significant (independent). The other two-factor ANOVA's show independent joint factors for three of the four combinations in each. There is no consistent pattern in these.

The patterns of first-order effects are depicted for each factor in Figure 5 and the analyses summarized in Table 10. There is a significant year effect in month=1 for both sexes for *D. melanogaster* (year=1 > year=2), but not for *D. simulans*. There is a significant month effect in *D. melanogaster* females in both years (month=1 > month=2). There is not other pattern of month effects. The effect of species is significant across all eight combinations (*D. melanogaster* > *D. simulans*). *D. simulans* shows a significant sex effect in all four of its year and month combinations (females > males).

The four-factor ANOVA for twelve hour desiccation resistance is summarized in Table 4. There is a marginally significant fourth-order interaction (p=0.06), implying that the joint effects of the four factors are independent. There are, however, some significant (dependent) third-order interactions, that are more appropriately addressed in the following lower order ANOVA tables.

The three-factor ANOVA's are summarized in Table 11. The third-order terms are significant in both analyses conditioned for species; and for year=1, month=1, and sex=male. In these cases, the effects of the other three factors are interdependent. The factors in the other analyses are all independent.

The two-factor ANOVA's are summarized in Table 12. The joint effects of year with month and with sex, and species with sex are interdependent across all combinations of the conditioning variables. The joint effects of month and sex are interdependent year=1 and for both species; but are independent for year=2 for both species. The joint effects of year and species and of month and species are independent for three of the four combinations.

The patterns of first-order effects are depicted for each factor in Figure 6 and summarized in Table 13. The effect of year is significant across all eight combinations (with no completely consistent pattern of one year greater than the other). The effect of month is significant across all combinations except for *D. simulans* females in year=2 (with no completely consistent pattern of one year greater than the other). The effect of species is significant across all eight combinations (*D. melanogaster* > *D. simulans*). There is a significant sex effect for both species in month=1 for year=1 (females > males). There is no other pattern of significant sex effects.

The four-factor ANOVA for twenty-four hour desiccation resistance is summarized in Table 4. There is no significant fourth-order interaction, implying that the joint effects of the four factors are independent. There are, however, some significant (dependent) third-order interactions, that are more appropriately addressed in the following lower order ANOVA tables.

The three-factor ANOVA's are summarized in Table 14. The third-order terms are significant in both analyses conditioned for species; and for year=2. In these cases, the effects of the other three factors are interdependent. The factors in the other analyses are all independent.

The two-factor ANOVA's are summarized in Table 15. The joint effects of year with month and species with sex are interdependent across all combinations of the conditioning variables. The joint effects of sex with year and with month are interdependent for three of the four combinations. The joint effects of month and species are independent for three of the four combinations. The joint effects of year and species are independent across all possible combinations of factors.

The patterns of first-order effects are depicted for each factor in Figure 7 and summarized in Table 16. The effect of year is significant across all eight combinations (with no completely consistent pattern but with most showing year=1 > year=2). The effect of month is significant across all eight combinations (with no completely consistent pattern but with most showing month=7 > month=1). The effect of species is significant across all eight combinations (*D. melanogaster* > *D. simulans*). The effect of sex is significant across all combinations except for *D. melanogaster* for month=7 in year=2 (females > males).

Analyses of variance for vagility consisted of two sets of factorial ANOVA's. One set for the four hour vagility readings, and one set for the 24 hour readings. Although these two sets of readings appear in the same tables, their analysis will be discussed separately.

The three-factor ANOVA for four hour vagility is summarized in Table 17. There is no significant third-order interaction, implying that the joint effects of the three factors are independent. There are, however, significant (interdependent) second-order interactions for year and month, which are more appropriately addressed in the following lower order ANOVA tables. Species shows a significant first-order (main) effect.

The three two-factor ANOVA's are summarized in Table 18. There are no significant interactions in these tables for four hour vagility when conditioned by year, month, or species. In these cases, the effects of year, month, and species are independent. There are significant main effects for species when conditioned either by year or month; and a significant year effect for month=1; and a significant month effect for year=2. These factors in these effects are interdependent.

The three one-factor ANOVA's are depicted in Figure 8 and summarized in Table 19. There is a single significant year effect in month=1 for *Drosophila melanogaster* (year=1 > year=2). There is a single significant month effect in year=2 for *D. melanogaster* (month=7 > month=1). Species shows significant effects for three of the four possibilities — for both months in both years except for month=1 in year=2. (In all three cases, *D. melanogaster* > *D. simulans*.)

The three-factor ANOVA for twenty-four hour vagility is summarized in Table 17. There is no significant third-order interaction, nor second-order interaction, implying that the joint effects of the three factors are independent. The second-order interaction for year and month is marginally significant (p=0.06) which is similar to the four hour vagility analysis. In the twenty-four hour vagility three-factor analysis, species (same as for the four hour analysis) and year show a significant first-order (main) effect.

The three two-factor ANOVA's are summarized in Table 18. There are no significant interactions for twenty-four hour vagility when conditioned by year, month, or species. In these cases, the effects of year, month, and species are independent. There are significant main effects for species (same as for the four hour analysis) when conditioned either by year or month. There is also a significant year effect for month=1. These factors in these effects are interdependent.

The three one-factor ANOVA's are depicted in Figure 9 and summarized in Table 19. There is a single significant year effect in month=1 for *Drosophila melanogaster* (year=1 > year=2). There are no significant month effects. Species shows significant effects for month=1 in both years. (In both cases, *D. melanogaster* > *D. simulans*.)

Analyses of variance for body size consisted of only one set of factorial ANOVA's. The four-factor ANOVA is summarized in Table 20. There is no significant fourth-order interaction, implying that the joint effects of the four factors are independent. There are, however, some significant third-order interactions, that are more appropriately addressed in the following lower order ANOVA tables.

The three-factor ANOVA's are summarized in Table 21. The third-order terms are significant only in the analyses conditioned on sex. In that case, the effects of year, month, and species are interdependent. The others are all independent.

The two-factor ANOVA's are summarized in Table 22. The joint effects of sex and both species and month are independent across all combinations of the conditioning variables. The joint effects of year and sex are also independent for three of the four combinations. Only in *D. simulans* for month=7 is the interaction significant. The joint effects of year and month are interdependent in both sexes of *D. simulans* but in neither sex in *D. melanogaster*. The joint effects of year and species are interdependent for both sexes in month=1 but in neither sex in month=7. The joint effects of month and species are interdependent for both sexes in year=2, but for neither in year=1.

The patterns of first-order effects are depicted for each factor in Figure 10 and summarized in Table 23. There is no single pattern of significant year effects across the eight combinations of conditioning variables. (For the three cases, year=1 > year=2.) *D. simulans* shows significant year effects for three out the four (all except males in month=7) combinations of month and sex. There is a significant month effect in both sexes of *D. simulans* for both years. (In both cases, month=1 > month=7.) The pattern is "nearly opposite" in *D. melanogaster* with no month effects except for that in males in year=1. The effect of species is significant across all conditioning variables. (All *D. melanogaster*> *D simulans* .) There is not a consistent pattern of significant sex effects across the eight combinations of the conditioning variables. There is a significant sex effect in *D. simulans* for month=1 in both years (females > males), but not for *D. melanogaster*. There is a significant sex effect in both species for month=7 (females > males) but not for month=1 in year=1.

The mean responses measure one facet of these phenotypes. Equally important to this study is a comparative evaluation of the variation in the traits. In particular, the level of genotypic variation is central to studies of this type. In this work, genotypic variation is based on the differential responses of the isofemale lines. Each isofemale line was raised and tested in the same environment. Repeated measurements of each line used flies drawn from different replicate cultures of each line. The actual level of isofemale line variation was estimated as the added component of variance due to isofemale lines (Rockwell, 1980). $\hat{S}_A^2$ is a measure of the expressed genotypic variance for each trait of a population and is an estimate of the phenotypic variance resulting from genotypic rather than environmental differences. The expressed genotypic variance reflects the broad genotypic structure of each trait for the populations under study. The expressed genotypic variance contains elements of the additive, dominance, and epistatic components of genetic variance.

The level of genotypic variation was compared for the traits in a pairwise fashion using a Monte Carlo method. This method assumes that the two variance components are equal and, therefore, have an expected ratio of 1. The probability that the observed ratio departs from this expectation due to chance alone is then evaluated by computer simulation (see Rockwell, 1980, for a full discussion of this method).

The estimates of genotypic variation for the traits are summarized in Tables 24—27 with respect to collection (year and season), species, sex, and time. Lower and upper 95% confidence limits based on Monte Carlo simulation are also included. Estimates that are marked significant indicate that the variation among isofemale lines was statistically greater than the pooled variation within isofemale lines, as evaluated with one-way ANOVA.

For desiccation resistance in males, significant $\hat{S}_A^2$ estimates were found for *D. melanogaster* for the July 1989 collection at 4 hours ($p \leq 0.01$), and for the January 1990 collection at 8 hours ($p \leq 0.05$). There were no significant $\hat{S}_A^2$ estimates for *D. melanogaster* females at the $p \leq 0.05$ level.

For desiccation resistance in males, significant $\hat{S}_A^2$ estimates were found for *D. simulans* for the January 1989 collection at 8 hours ($p \leq 0.05$), and at 12 hours ($p \leq 0.01$). *D. simulans* females were significant for the January 1989 collection at 8 hours

($p \leq 0.01$), and marginally significant for the July 1988 collection at 24 hours ($p=0.0574$) and the July 1989 collection at 12 hours ($p=0.0527$).

For vagility in males (only), significant $\hat{S}_A^2$ estimates were found for *D. melanogaster* for the July 1989 collection for 24 hours ($p \leq 0.01$). The July 1988 collection for 4 hours was marginally significant at the $p=0.0525$ level. For *D. simulans* in males, significant $\hat{S}_A^2$ estimates were found for the January 1989 collection for 4 hours and 24 hours ($p \leq 0.01$).

For body size in males and in females, significant $\hat{S}_A^2$ estimates were found for *D. melanogaster* for all four collections. All were significant at the $p \leq 0.01$ level, except for the July 1988 female collection which was significant at the $p \leq 0.05$ level.

For body size in males and in females, significant $\hat{S}_A^2$ estimates were found for *D. simulans* for all four collections ($p \leq 0.01$) except for the July 1988 female collection.

For desiccation resistance, comparisons within species were first made among collections for common time readings and sex. Sexes were compared for common time readings and collections. Between species comparisons were made for common time readings, collection, and sex.

In *D. melanogaster*, there were significant differences in expressed genotypic variance of desiccation resistance of males between the January 1989 and July 1989 collections and between the January 1989 and January 1990 collections for both the 8 and 12 hour measurements. Significant expressed genotypic variance differences were also found between the July 1988 and the January 1990 collections at 4 hours, and between the July 1989 and the January 1990 collections at 4 hours. There were no differences among the collections in expressed genotypic variance of females. There were no significant differences between the sexes in expressed genotypic variance for any of the collections.

In *D. simulans*, there were no significant differences in expressed genotypic variance among the collections for desiccation resistance of males. For females there were significant differences between the July 1988 and January 1990 and between the January

1989 and January 1990 collections at 4 hours. There were no differences between the sexes.

Between *D. melanogaster* and *D. simulans*, there were significant differences in expressed genotypic variance of desiccation resistance in males for the January 1989 collection at both 8 and 12 hours and for the January 1990 collection at 4 hours. Significant differences in females existed for the July 1988 collection at both 4 and 24 hours and for the January 1990 collection at 8 hours.

For vagility (males only), comparisons were made among collections for common time readings within each species. Between species comparisons were made for common time reading and collection. There were no significant differences found in expressed genotypic variance of vagility in males for any of the comparisons within *D. melanogaster* or *D. simulans* or between the two species.

For body size, comparisons were first made among the collections for both sexes of the species. Sexes were then compared for each collection of the species. Between species comparisons were made for common collection and sex. There were no significant differences found in expressed genotypic variance of body size in males and females for any of the comparisons within *D. melanogaster* or *D. simulans* or between the two species.

Correlation analyses were performed to examine the relationship between desiccation resistance, vagility, and body size. Each desiccation resistance time reading (4, 8, 12, and 24 hours) was evaluated with each vagility time reading (4 and 24 hours) and with the body size measurement. Each vagility reading was also evaluated with body size. (Females were not used in the vagility experiments and, therefore, are not part of the vagility analyses.) The correlation coefficient (r), probability of its difference from zero (p), and coefficient of determination (r^2) were determined. All analyses were performed for males and females separately.

Correlation analyses for each sex for the traits were first determined by species to give an overall view of any existing patterns. To unmask any statistically significant results, further analyses were performed for each year/species/sex and month/species/sex combination.

Correlation analyses for each species for each sex are summarized in Table 28. There were no statistically significant correlations for *Drosophila melanogaster* males. For *D. melanogaster* females, body size and desiccation resistance at 12 hours are negatively correlated. For *Drosophila simulans* males, desiccation resistance (8 hours) and vagility (24 hours) are negatively correlated as are body size and desiccation resistance at both 12 and 24 hours. For *D. simulans* females, body size and desiccation resistance at both 12 and 24 hours are negatively correlated.

Correlation analyses for each year with each species for each sex are summarized in Table 29. For *D. melanogaster* males, desiccation resistance (8 hours) and vagility (4 hours) are negatively correlated. For *D. simulans* males, all comparisons for desiccation resistance (8 hours) and vagility (4 and 24 hours), and for desiccation resistance (8 hours) and body size are negatively correlated. Body size is also negatively correlated with desiccation resistance at 12 and 24 hours. Correlation analyses for year/species/sex for year=1 show no statistically significant results for female *D. melanogaster* or *D. simulans.*

For year=2, for *D. melanogaster* males, desiccation resistance at 4 hours and body size are negatively correlated. For *D. melanogaster* females, desiccation resistance (4, 8, and 12 hours) and body size are also negatively correlated. For *D. simulans* males, desiccation resistance (12 and 24 hours) and body size are negatively correlated. *D. simulans* females show one negative correlation for desiccation resistance at 24 hours and body size .

Correlation analyses for each month with each species for each sex are summarized in Table 30. For month=7, for *D. melanogaster* males, desiccation resistance at 4 hours and body size are negatively correlated. For month=7, for *D. melanogaster* females, desiccation resistance (8 and 12 hours) and body size are negatively correlated. *D. simulans* males show no statistically significant results. For *D. simulans* females, desiccation resistance (12 and 24 hours) and body size are negatively correlated.

For month=1, for *D. melanogaster* males, desiccation resistance (4 hours) and vagility (4 hours), and for desiccation resistance (4 and 12 hours) and vagility (24 hours) are negatively correlated. For month=1, *D. melanogaster* females show no significant results. For *D. simulans* males, desiccation resistance (8 hours) and vagility (4 and 24 hours), desiccation resistance (12 hours) and vagility (4 hours), and desiccation resistance (12 hours) and body size, and desiccation resistance (24 hours) and body size are all negatively correlated. For *D. simulans* females, desiccation resistance at 24 hours and body size are negatively correlated.

Table 1. Means ± standard errors of desiccation resistance proportions of *Drosophila* species.

MALES

	D. melanogaster				*D. simulans*			
Date	4 Hours	8 Hours	12 Hours	24 Hours	4 Hours	8 Hours	12 Hours	24 Hours
8807	0.977±0.006	0.903±0.017	0.806±0.024	0.075±0.023	0.877±0.019	0.619±0.029	0.334±0.029	0.000±0.000
8901	0.993±0.004	0.981±0.005	0.849±0.020	0.038±0.011	0.910±0.016	0.544±0.030	0.189±0.024	0.002±0.001
8907	0.990±0.005	0.952±0.014	0.898±0.022	0.054±0.010	0.973±0.009	0.693±0.029	0.325±0.034	0.000±0.000
9001	0.997±0.001	0.959±0.013	0.849±0.026	0.069±0.010	0.929±0.015	0.565±0.037	0.347±0.041	0.000±0.000

FEMALES

	D. melanogaster				*D. simulans*			
Date	4 Hours	8 Hours	12 Hours	24 Hours	4 Hours	8 Hours	12 Hours	24 Hours
8807	0.983±0.006	0.951±0.010	0.889±0.017	0.310±0.034	0.967±0.008	0.836±0.022	0.625±0.032	0.008±0.004
8901	0.999±0.001	0.995±0.002	0.963±0.009	0.261±0.027	0.986±0.006	0.914±0.019	0.663±0.036	0.019±0.014
8907	0.993±0.003	0.970±0.010	0.924±0.020	0.364±0.033	0.989±0.003	0.904±0.016	0.598±0.035	0.002±0.001
9001	0.998±0.001	0.977±0.007	0.911±0.016	0.274±0.031	0.966±0.011	0.771±0.035	0.579±0.039	0.003±0.002

N.B.: These data have been published in Rockwell, R.F., M.E. de la Rosa, J. Guzmán, M.J. Laverde, L. Levine, and O. Olvera. 1991. A temporal study of desiccation resistance of sibling *Drosophila* species from Laguna Verde, Veracruz, Mexico. *American Midland Naturalist* **126**(2):338-344.

Table 2. Means ± standard errors of vagility scores of *Drosophila* species.

MALES (only)

	D. melanogaster		*D. simulans*	
Date	4 Hours	24 Hours	4 Hours	24 Hours
8807	0.301±0.025	0.393±0.024	0.184±0.018	0.326±0.025
8901	0.295±0.023	0.446±0.019	0.189±0.022	0.353±0.026
8907	0.340±0.034	0.415±0.032	0.209±0.025	0.303±0.024
9001	0.219±0.022	0.348±0.022	0.160±0.018	0.269±0.017

N.B.: These data have been published in Olvera, O., R.F. Rockwell, M.E. de la Rosa, J. Guzmán, M.J. Laverde, and L. Levine. 1993. Studies of Sibling *Drosophila* species from Laguna Verde, Veracruz, Mexico: V. Temporal Stability in Dispersive Behavior. *Southwestern Naturalist* 38(1):15-18.

Table 3. Means ± standard errors of body size measurements (in mm) of *Drosophila* species.

MALES

Date	*D. melanogaster*	*D. simulans*
8807	1.428±0.008	1.278±0.011
8901	1.418±0.011	1.301±0.008
8907	1.328±0.013	1.224±0.013
9001	1.436±0.013	1.247±0.011

FEMALES

Date	*D. melanogaster*	*D. simulans*
8807	1.583±0.011	1.398±0.012
8901	1.597±0.012	1.433±0.012
8907	1.500±0.013	1.375±0.011
9001	1.646±0.016	1.389±0.009

Table 4. Four-factor analyses of variance of desiccation resistance (4, 8, 12, and 24 hours) of *Drosophila* species.

		YEAR, MONTH, SPECIES, SEX			
SOURCE	df	4 Hours	8 Hours	12 Hours	24 Hours
YEAR	1	0.016**	0.029*	0.026	0.038**
MONTH	1	0.001	0.005	0.306**	0.051**
SPECIES	1	0.070**	2.118**	7.349**	1.247**
SEX	1	0.030**	0.370**	0.072**	0.068**
YEAR*MONTH	1	0.009*	0.001	1.625**	0.583**
YEAR*SPECIES	1	0.005*	<0.001	0.001	0.007
YEAR*SEX	1	0.012**	0.017	0.002	0.055**
MONTH*SPECIES	1	0.002	0.098**	0.016	0.019**
MONTH*SEX	1	<0.001	0.029*	0.173**	0.053**
SPECIES*SEX	1	0.026**	0.511**	0.607**	0.555**
YEAR*MONTH*SPECIES	1	0.006**	0.016	0.052*	<0.001
YEAR*MONTH*SEX	1	<0.001	0.023*	0.509**	0.200**
YEAR*SPECIES*SEX	1	0.007**	0.013	0.014	0.004
MONTH*SPECIES*SEX	1	<0.001	0.021$^{\#}$	0.004	0.010
YEAR*MONTH*SPECIES*SEX	1	0.001	0.023*	0.033$^{\#}$	0.004
error	144	0.001	0.006	0.009	0.003

**$p \leq 0.01$

*$p \leq 0.05$

$^{\#}p=0.06$

N.B.: Columnar values are the mean squares.
All scores were corrected using the Or.-R laboratory strain as a yoked control.
All sources were tested over the MS error.

Table 5. Three-factor analyses of variance of desiccation resistance (4 hours) of *Drosophila* species.

		MONTH SPECIES SEX by Year		YEAR SPECIES SEX by Month		YEAR MONTH SEX by Species		YEAR MONTH SPECIES by Sex	
Source	df	Year=1	Year=2	Month=7	Month=1	*D. mel.*	*D. sim.*	male	female
YEAR	1	▨	▨	0.024**	<0.001	0.001*	0.020**	0.028**	<0.001
MONTH	1	0.009**	0.002	▨	▨	0.004**	<0.001	0.001	<0.001
SPECIES	1	0.057**	0.019**	0.024**	0.049**	▨	▨	0.091**	0.005**
SEX	1	0.040**	0.002	0.017**	0.013**	<0.001	0.056**	▨	▨
YR*MO	1	▨	▨	▨	▨	0.001	0.015**	0.006	0.003**
YR*SP	1	▨	▨	0.011**	<0.001	▨	▨	0.012**	<0.001
YR*SEX	1	▨	▨	0.008**	0.004*	<0.001	0.019**	▨	▨
MO*SP	1	0.001	0.008**	▨	▨	▨	▨	0.001	0.001
MO*SEX	1	<0.001	<0.001	▨	▨	<0.001	<0.001	▨	▨
SP*SEX	1	0.030**	0.003*	0.012**	0.014**	▨	▨	▨	▨
YR*MO*SP	1	▨	▨	▨	▨	▨	▨	0.006*	0.001
YR*MO*SEX	1	▨	▨	▨	▨	<0.001	0.001	▨	▨
YR*SP*SEX	1	▨	▨	0.006**	0.001	▨	▨	▨	▨
MO*SP*SEX	1	<0.001	0.001	▨	▨	▨	▨	▨	▨
error	72	0.001	0.007	0.001	0.001	<0.001	0.002	0.001	<0.001

**p≤0.01
*p≤0.05
#p=0.06

N.B.: Columnar values are the mean squares.
Shading indicates sources not possible for various factor combinations.
All scores were corrected using the Or.-R laboratory strain as a yoked control.
All sources were tested over the MS error.

Table 6. Two-factor analyses of variance of desiccation resistance (4 hours) of *Drosophila* species.

YEAR, MONTH by Species, Sex

		D. melanogaster		*D. simulans*	
SOURCE	df	male	female	male	female
YEAR	1	0.002*	<0.001	0.038**	<0.001
MONTH	1	0.003**	0.001*	<0.001	<0.001
YEAR*MONTH	1	<0.001	<0.001	0.012*	0.004**
error	36	<0.001	<0.001	0.002	0.001

YEAR, SPECIES by Month, Sex

		Month=7		Month-1	
SOURCE	df	male	female	male	female
YEAR	1	0.029**	0.002*	0.004	0.001
SPECIES	1	0.035**	0.001	0.058**	0.005**
YEAR*SPECIES	1	0.017**	<0.001	0.001	0.001
error	36	0.001	<0.001	0.001	<0.001

YEAR, SEX by Month, Species

		Month=7		Month-1	
SOURCE	df	*D. mel.*	*D. sim.*	*D. mel.*	*D. sim.*
YEAR	1	0.001	0.035**	<0.001**	<0.001
SEX	1	<0.001	0.029**	<0.001	0.027**
YEAR*SEX	1	<0.001	0.014**	<0.001**	0.006
error	36	<0.001	0.001	<0.001	0.002

Table 6. continued.

MONTH, SPECIES by Year, Sex

		Year=1		Year=2	
SOURCE	df	male	female	male	female
MONTH	1	0.006*	0.003**	0.001	0.001
SPECIES	1	0.085**	0.002*	0.018**	0.003**
MONTH*SPECIES	1	0.001	<0.001	0.006*	0.002*
error	36	0.002	<0.001	0.001	<0.001

MONTH, SEX by Year, Species

		YEAR=1		YEAR=2	
SOURCE	df	*D. mel.*	*D. sim.*	*D. mel.*	*D. sim.*
MONTH	1	0.003**	0.007*	0.001**	0.009*
SEX	1	<0.001	0.069**	<0.001	0.005
MONTH*SEX	1	<0.001	0.001	<0.001	<0.001
error	36	<0.001	0.002	<0.001	0.001

SPECIES, SEX by Year, Month

		Year=1		Year=2	
SOURCE	df	Month=7	Month=1	Month=7	Month=1
SPECIES	1	0.034**	0.023**	0.001	0.025**
SEX	1	0.023**	0.017**	0.001	0.001
SPECIES*SEX	1	0.018**	0.012**	0.001	0.003
error	36	0.001	0.001	<0.001	0.001

$^{**}p \leq 0.01$
$^{*}p \leq 0.05$
#p=0.06

N.B.: Columnar values are the mean squares.
All scores were corrected using the Or.-R laboratory strain as a yoked control.
All sources were tested over the MS error.

Table 7. One-factor analyses of variance of desiccation resistance (4 hours) of *Drosophila* species.

YEAR by Month, Species, Sex

		Month=7				Month=1			
		D. melanogaster		*D. simulans*		*D. melanogaster*		*D. simulans*	
SOURCE	df	male	female	male	female	male	female	male	female
YEAR	1	0.001	<0.001	0.046**	0.002*	0.001**	<0.001	0.004	0.002
error	18	0.001	<0.001	0.002	<0.001	<0.001	<0.001	0.002	0.001

MONTH by Year, Species, Sex

		Year=1				Year=2			
		D. melanogaster		*D. simulans*		*D. melanogaster*		*D. simulans*	
SOURCE	df	male	female	male	female	male	female	male	female
MONTH	1	0.001	0.001#	0.006	0.002	0.001*	<0.001	0.006	0.003
error	18	<0.001	<0.001	0.003	0.001	<0.001	<0.001	0.002	0.001

SPECIES by Year, Month, Sex

		Year=1				Year=2			
		Month=7		Month=1		Month=7		Month=1	
SOURCE	df	male	female	male	female	male	female	male	female
SPECIES	1	0.051**	0.001	0.035*	0.001*	0.002	<0.001	0.023**	0.005*
error	18	0.002	0.001	0.001	<0.001	0.001	<0.001	0.002	0.001

SEX by Year, Month, Species

		Year=1				Year=2			
		Month=7		Month=1		Month=7		Month=1	
SOURCE	df	*D. mel.*	*D. sim.*	*D. mel.*	*D. sim.*	*D. mel.*	*D. sim.*	*D. mel.*	*D. sim.*
SEX	1	<0.001	0.041**	<0.001	0.029**	<0.001	0.001	<0.001**	0.004
error	18	0.001	0.002	<0.001	0.001	<0.001	0.001	<0.001	0.002

**$p \leq 0.01$
*$p \leq 0.05$
#$p = 0.06$

N.B.: Columnar values are the mean squares.
All scores were corrected using the Or.-R laboratory strain as a yoked control.
All sources were tested over the MS error.

Table 8. Three-factor analyses of variance of desiccation resistance (8 hours) of *Drosophila* species.

		MONTH SPECIES SEX by Year		YEAR SPECIES SEX by Month		YEAR MONTH SEX by Species		YEAR MONTH SPECIES by Sex	
Source	df	Year=1	Year=2	Month=7	Month=1	*D. mel.*	*D. sim.*	male	female
YEAR	1	▨	▨	0.020*	0.010	0.016**	0.013	0.045*	0.001
MONTH	1	0.001	0.005	▨	▨	0.030**	0.073**	0.028*	0.005
SPECIES	1	1.048**	1.070**	0.625**	1.563**	▨	▨	2.355**	0.274**
SEX	1	0.271**	0.115**	0.096**	0.302**	0.006	0.875**	▨	▨
YR*MO	1	▨	▨	▨	▨	0.004	0.012	0.007	0.017$^{\#}$
YR*SP	1	▨	▨	0.007	0.009	▨	▨	0.006	0.007
YR*SEX	1	▨	▨	<0.001	0.039*	<0.001	0.029	▨	▨
MO*SP	1	0.018	0.096**	▨	▨	▨	▨	0.105**	0.014
MO*SEX	1	0.051**	<0.001	▨	▨	<0.001	0.049*	▨	▨
SP*SEX	1	0.343**	0.181**	0.163**	0.369**	▨	▨	▨	▨
YR*MO*SP	1	▨	▨	▨	▨	▨	▨	<0.001	0.038**
YR*MO*SEX	1	▨	▨	▨	▨	<0.001	0.046*	▨	▨
YR*SP*SEX	1	▨	▨	0.001	0.035*	▨	▨	▨	▨
MO*SP*SEX	1	0.044**	<0.001	▨	▨	▨	▨	▨	▨
error	72	0.005	0.007	0.005	0.002	0.002	0.010	0.007	0.004

**p≤0.01
*p≤0.05
$^{\#}$p=0.06

N.B.: Columnar values are the mean squares.
Shading indicates sources not possible for various factor combinations.
All scores were corrected using the Or.-R laboratory strain as a yoked control.
All sources were tested over the MS error.

Table 9. Two-factor analyses of variance of desiccation resistance (8 hours) of *Drosophila* species.

YEAR, MONTH by Species, Sex

		D. melanogaster		*D. simulans*	
SOURCE	df	male	female	male	female
YEAR	1	0.009#	0.007**	0.041	0.002
MONTH	1	0.012*	0.018**	0.121**	0.001
YEAR*MONTH	1	0.002	0.002	0.005	0.053**
error	36	0.002	0.001	0.012	0.008

YEAR, SPECIES by Month, Sex

		Month=7		Month-1	
SOURCE	df	male	female	male	female
YEAR	1	0.008	0.013*	0.044*	0.005
SPECIES	1	0.734**	0.082**	1.726**	0.207**
YEAR*SPECIES	1	0.002	0.006	0.005	0.040*
error	36	0.007	0.003	0.008	0.006

YEAR, SEX by Month, Species

		Month=7		Month-1	
SOURCE	df	*D. mel.*	*D. sim.*	*D. mel.*	*D. sim.*
YEAR	1	0.002	0.025	0.0184**	<0.001
SEX	1	0.004	0.255**	0.002	0.670**
YEAR*SEX	1	<0.001	0.001	<0.001	0.074*
error	36	0.002	0.007	0.001	0.013

Table 9. continued.

MONTH, SPECIES by Year, Sex

		Year=1		Year=2	
SOURCE	df	male	female	male	female
MONTH	1	0.032*	0.020*	0.004	0.002
SPECIES	1	1.296**	0.096**	1.064**	0.186**
MONTH*SPECIES	1	0.059**	0.003	0.046*	0.050**
error	36	0.007	0.003	0.008	0.006

MONTH, SEX by Year, Species

		YEAR=1		YEAR=2	
SOURCE	df	*D. mel.*	*D. sim.*	*D. mel.*	*D. sim.*
MONTH	1	0.006*	0.013	0.028**	0.072**
SEX	1	0.002	0.613**	0.004	0.292**
MONTH*SEX	1	<0.001	0.095**	<0.001	<0.001
error	36	0.001	0.009	0.002	0.011

SPECIES, SEX by Year, Month

		Year=1		Year=2	
SOURCE	df	Month=7	Month=1	Month=7	Month=1
SPECIES	1	0.397**	0.670**	0.263**	0.903**
SEX	1	0.043**	0.279**	0.053**	0.062**
SPECIES*SEX	1	0.071**	0.317**	0.093**	0.088**
error	36	0.004	0.007	0.006	0.008

**$p \leq 0.01$
*$p \leq 0.05$
#$p = 0.06$

N.B.: Columnar values are the mean squares.
All scores were corrected using the Or.-R laboratory strain as a yoked control.
All sources were tested over the MS error.

Table 10. One-factor analyses of variance of desiccation resistance (8 hours) of *Drosophila* species.

YEAR by Month, Species, Sex

		Month=7				Month=1			
		D. melanogaster		*D. simulans*		*D. melanogaster*		*D. simulans*	
SOURCE	df	male	female	male	female	male	female	male	female
YEAR	1	0.001	0.001	0.008	0.018*	0.010*	0.008**	0.038	0.036
error	18	0.003	0.001	0.010	0.004	0.002	<0.001	0.014	0.012

MONTH by Year, Species, Sex

		Year=1				Year=2			
		D. melanogaster		*D. simulans*		*D. melanogaster*		*D. simulans*	
SOURCE	df	male	female	male	female	male	female	male	female
MONTH	1	0.002	0.004**	0.089*	0.019	0.012	0.017**	0.038	0.035
error	18	0.001	<0.001	0.012	0.006	0.003	0.001	0.012	0.010

SPECIES by Year, Month, Sex

		Year=1				Year=2			
		Month=7		Month=1		Month=7		Month=1	
SOURCE	df	male	female	male	female	male	female	male	female
SPECIES	1	0.401**	0.066**	0.953**	0.033**	0.334**	0.022**	0.777**	0.214**
error	18	0.005	0.003	0.009	0.004	0.009	0.003	0.007	0.009

SEX by Year, Month, Species

		Year=1				Year=2			
		Month=7		Month=1		Month=7		Month=1	
SOURCE	df	*D. mel.*	*D. sim.*	*D. mel.*	*D. sim.*	*D. mel.*	*D. sim.*	*D. mel.*	*D. sim.*
SEX	1	0.002	0.113**	0.001	0.595**	0.003	0.143**	0.001	0.149**
error	18	0.002	0.005	<0.001	0.013	0.003	0.009	0.002	0.013

**$p \leq 0.01$

*$p \leq 0.05$

#$p = 0.06$

N.B.: Columnar values are the mean squares.
All scores were corrected using the Or.-R laboratory strain as a yoked control.
All sources were tested over the MS error.

Table 11. Three-factor analyses of variance of desiccation resistance (12 hours) of *Drosophila* species.

		MONTH SPECIES SEX by Year		YEAR SPECIES SEX by Month		YEAR MONTH SEX by Species		YEAR MONTH SPECIES by Sex	
Source	df	Year=1	Year=2	Month=7	Month=1	*D. mel.*	*D. sim.*	male	female
YEAR	1	▨	▨	1.031**	0.620**	0.009	0.018	0.007	0.021
MONTH	1	1.670**	0.261**	▨	▨	0.091**	0.230**	0.469**	0.009
SPECIES	1	3.595**	3.756**	3.342**	4.024**	▨	▨	6.091**	1.866**
SEX	1	0.048**	0.026	0.011	0.234**	0.131**	0.549**	▨	▨
YR*MO	1	▨	▨	▨	▨	0.547**	1.130**	1.977**	0.158**
YR*SP	1	▨	▨	0.033*	0.020	▨	▨	0.004	0.011
YR*SEX	1	▨	▨	0.226**	0.285**	0.003	0.013	▨	▨
MO*SP	1	0.063**	0.005	▨	▨	▨	▨	0.018	0.002
MO*SEX	1	0.638**	0.044*	▨	▨	0.063**	0.113**	▨	▨
SP*SEX	1	0.403**	0.219**	0.259**	0.352**	▨	▨	▨	▨
YR*MO*SP	1	▨	▨	▨	▨	▨	▨	0.084**	0.001
YR*MO*SEX	1	▨	▨	▨	▨	0.142**	0.400**	▨	▨
YR*SP*SEX	1	▨	▨	0.002	0.045*	▨	▨	▨	▨
MO*SP*SEX	1	0.029*	0.007	▨	▨	▨	▨	▨	▨
error	72	0.007	0.010	0.008	0.010	0.005	0.013	0.009	0.009

$^{**}p \leq 0.01$
$^{*}p \leq 0.05$
$^{\#}p = 0.06$

N.B.: Columnar values are the mean squares.
Shading indicates sources not possible for various factor combinations.
All scores were corrected using the Or.-R laboratory strain as a yoked control.
All sources were tested over the MS error.

Table 12. Two-factor analyses of variance of desiccation resistance (12 hours) of *Drosophila* species.

YEAR, MONTH by Species, Sex

		D. melanogaster		*D. simulans*	
SOURCE	df	male	female	male	female
YEAR	1	0.011	0.001	<0.001	0.031
MONTH	1	0.153**	0.001	0.333**	0.010
YEAR*MONTH	1	0.623**	0.066**	1.438**	0.093*
error	36	0.006	0.003	0.011	0.015

YEAR, SPECIES by Month, Sex

		Month=7		Month-1	
SOURCE	df	male	female	male	female
YEAR	1	1.111**	0.146**	0.873**	0.032#
SPECIES	1	2.730**	0.870**	3.379**	0.998**
YEAR*SPECIES	1	0.026#	0.010	0.062*	0.003
error	36	0.007	0.009	0.010	0.009

YEAR, SEX by Month, Species

		Month=7		Month-1	
SOURCE	df	*D. mel.*	*D. sim.*	*D. mel.*	*D. sim.*
YEAR	1	0.347**	0.718**	0.209**	0.431**
SEX	1	0.188**	0.082**	0.006	0.580**
YEAR*SEX	1	0.093**	0.135**	0.052**	0.278**
error	36	0.005	0.011	0.004	0.015

Table 12. continued.

MONTH, SPECIES by Year, Sex

		Year=1		Year=2	
SOURCE	df	male	female	male	female
MONTH	1	2.186**	0.122**	0.260**	0.045$^{\#}$
SPECIES	1	3.202**	0.796**	2.894**	1.081**
MONTH*SPECIES	1	0.089**	0.003	0.013	<0.001
error	36	0.008	0.006	0.009	0.012

MONTH, SEX by Year, Species

		YEAR=1		YEAR=2	
SOURCE	df	*D. mel.*	*D. sim.*	*D. mel.*	*D. sim.*
MONTH	1	0.543**	1.190**	0.096**	0.170**
SEX	1	0.086**	0.364**	0.047**	0.197**
MONTH*SEX	1	0.197**	0.469**	0.008	0.044
error	36	0.003	0.012	0.006	0.014

SPECIES, SEX by Year, Month

		Year=1		Year=2	
SOURCE	df	Month=7	Month=1	Month=7	Month=1
SPECIES	1	1.354**	2.304**	2.021**	1.740**
SEX	1	0.168**	0.518**	0.069*	0.001
SPECIES*SEX	1	0.108**	0.324**	0.153**	0.073**
error	36	0.005	0.009	0.011	0.010

**p≤0.01
*p≤0.05
$^{\#}$p=0.06

N.B.: Columnar values are the mean squares.
All scores were corrected using the Or.-R laboratory strain as a yoked control.
All sources were tested over the MS error.

Table 13. One-factor analyses of variance of desiccation resistance (12 hours) of *Drosophila* species.

YEAR by Month, Species, Sex

		Month=7				Month=1			
		D. melanogaster		*D. simulans*		*D. melanogaster*		*D. simulans*	
SOURCE	df	male	female	male	female	male	female	male	female
YEAR	1	0.399**	0.041**	0.738**	0.115**	0.235**	0.026**	0.700**	0.008
error	18	0.005	0.005	0.008	0.014	0.007	0.001	0.013	0.016

MONTH by Year, Species, Sex

		Year=1				Year=2			
		D. melanogaster		*D. simulans*		*D. melanogaster*		*D. simulans*	
SOURCE	df	male	female	male	female	male	female	male	female
MONTH	1	0.697**	0.043**	1.577**	0.082**	0.079**	0.025*	0.193**	0.021
error	18	0.004	0.002	0.012	0.011	0.009	0.004	0.009	0.019

SPECIES by Year, Month, Sex

		Year=1				Year=2			
		Month=7		Month=1		Month=7		Month=1	
SOURCE	df	male	female	male	female	male	female	male	female
SPECIES	1	1.112**	0.349**	2.178**	0.450**	1.644**	0.531**	1.263**	0.550**
error	18	0.006	0.004	0.011	0.008	0.008	0.014	0.010	0.010

SEX by Year, Month, Species

		Year=1				Year=2			
		Month=7		Month=1		Month=7		Month=1	
SOURCE	df	*D. mel.*	*D. sim.*	*D. mel.*	*D. sim.*	*D. mel.*	*D. sim.*	*D. mel.*	*D. sim.*
SEX	1	0.272**	0.003	0.011*	0.830**	0.008	0.214**	0.047*	0.028
error	18	0.004	0.007	0.002	0.016	0.006	0.016	0.007	0.013

**p≤0.01
*p≤0.05
$^{\#}$p=0.06

N.B.: Columnar values are the mean squares.
All scores were corrected using the Or.-R laboratory strain as a yoked control.
All sources were tested over the MS error.

Table 14. Three-factor analyses of variance of desiccation resistance (24 hours) of *Drosophila* species.

		MONTH SPECIES SEX by Year		YEAR SPECIES SEX by Month		YEAR MONTH SEX by Species		YEAR MONTH SPECIES by Sex	
Source	df	Year=1	Year=2	Month=7	Month=1	*D. mel.*	*D. sim.*	male	female
YEAR	1	▨	▨	0.460**	0.161**	0.007	0.038**	0.001	0.093**
MONTH	1	0.145**	0.490**	▨	▨	0.004	0.066**	<0.001	0.105**
SPECIES	1	0.536**	0.717**	0.787**	0.479**	▨	▨	0.069**	1.733**
SEX	1	<0.001	0.123**	0.121**	<0.001	0.117**	0.507**	▨	▨
YR*MO	1	▨	▨	▨	▨	0.305**	0.278**	0.050**	0.733**
YR*SP	1	▨	▨	0.002	0.005	▨	▨	<0.001	0.010
YR*SEX	1	▨	▨	0.233**	0.023**	0.015	0.044**	▨	▨
MO*SP	1	0.012	0.007$^{\#}$	▨	▨	▨	▨	0.001	0.028*
MO*SEX	1	0.023*	0.230**	▨	▨	0.009	0.055**	▨	▨
SP*SEX	1	0.233**	0.326**	0.358**	0.208**	▨	▨	▨	▨
YR*MO*SP	1	▨	▨	▨	▨	▨	▨	0.004	0.001
YR*MO*SEX	1	▨	▨	▨	▨	0.073**	0.132**	▨	▨
YR*SP*SEX	1	▨	▨	0.008	<0.001	▨	▨	▨	▨
MO*SP*SEX	1	0.001	0.014**	▨	▨	▨	▨	▨	▨
error	72	0.004	0.002	0.003	0.003	0.006	<0.001	0.001	0.005

**$p \leq 0.01$
*$p \leq 0.05$
$^{\#}p = 0.06$

N.B.: Columnar values are the mean squares.
Shading indicates sources not possible for various factor combinations.
All scores were corrected using the Or.-R laboratory strain as a yoked control.
All sources were tested over the MS error.

Table 15. Two-factor analyses of variance of desiccation resistance (24 hours) of *Drosophila* species.

YEAR, MONTH by Species, Sex

		D. melanogaster		*D. simulans*	
SOURCE	df	male	female	male	female
YEAR	1	0.001	0.021	<0.001**	0.083**
MONTH	1	<0.001	0.012	<0.001**	0.121**
YEAR*MONTH	1	0.040**	0.338**	0.013**	0.397**
error	36	0.002	0.009	<0.001	0.001

YEAR, SPECIES by Month, Sex

		Month=7		Month-1	
SOURCE	df	male	female	male	female
YEAR	1	0.019**	0.674**	0.032**	0.152**
SPECIES	1	0.042**	1.103**	0.028**	0.659**
YEAR*SPECIES	1	0.001	0.009	0.003#	0.002
error	36	0.002	0.005	0.001	0.005

YEAR, SEX by Month, Species

		Month=7		Month-1	
SOURCE	df	*D. mel.*	*D. sim.*	*D. mel.*	*D. sim.*
YEAR	1	0.201**	0.261**	0.111**	0.055**
SEX	1	0.032*	0.448**	0.094**	0.114**
YEAR*SEX	1	0.077**	0.165**	0.011	0.012**
error	36	0.006	<0.001	0.005	0.001

Table 15. continued.

MONTH, SPECIES by Year, Sex

		Year=1		Year=2	
SOURCE	df	male	female	male	female
MONTH	1	0.026**	0.142**	0.024**	0.696**
SPECIES	1	0.031**	0.738**	0.038**	1.005**
MONTH*SPECIES	1	0.004	0.009	0.001	0.021*
error	36	0.002	0.007	0.001	0.003

MONTH, SEX by Year, Species

		YEAR=1		YEAR=2	
SOURCE	df	*D. mel.*	*D. sim.*	*D. mel.*	*D. sim.*
MONTH	1	0.120**	0.037**	0.189**	0.308**
SEX	1	0.108**	0.126**	0.024*	0.425**
MONTH*SEX	1	0.016	0.008**	0.065**	0.179**
error	36	0.008	0.001	0.004	<0.001

SPECIES, SEX by Year, Month

		Year=1		Year=2	
SOURCE	df	Month=7	Month=1	Month=7	Month=1
SPECIES	1	0.355**	0.194**	0.434**	0.290**
SEX	1	0.009	0.015*	0.345**	0.008
SPECIES*SEX	1	0.128**	0.105**	0.238**	0.103**
error	36	0.005	0.003	0.002	0.002

$^{**}p \leq 0.01$
$^{*}p \leq 0.05$
$^{\#}p = 0.06$

N.B.: Columnar values are the mean squares.
All scores were corrected using the Or.-R laboratory strain as a yoked control.
All sources were tested over the MS error.

Table 16. One-factor analyses of variance of desiccation resistance (24 hours) of *Drosophila* species.

YEAR by Month, Species, Sex

		Month=7				Month=1			
		D. melanogaster		*D. simulans*		*D. melanogaster*		*D. simulans*	
SOURCE	df	male	female	male	female	male	female	male	female
YEAR	1	0.015*	0.263**	0.006**	0.421**	0.026**	0.096**	0.008**	0.059**
error	18	0.003	0.010	<0.001	<0.001	0.001	0.009	<0.001	0.001

MONTH by Year, Species, Sex

		Year=1				Year=2			
		D. melanogaster		*D. simulans*		*D. melanogaster*		*D. simulans*	
SOURCE	df	male	female	male	female	male	female	male	female
MONTH	1	0.024*	0.111**	0.005**	0.040**	0.016**	0.238**	0.009**	0.478**
error	18	0.004	0.012	<0.001	0.001	0.001	0.007	<0.001	<0.001

SPECIES by Year, Month, Sex

		Year=1				Year=2			
		Month=7		Month=1		Month=7		Month=1	
SOURCE	df	male	female	male	female	male	female	male	female
SPECIES	1	0.028**	0.455**	0.007**	0.292**	0.015**	0.657**	0.024**	0.369**
error	18	0.003	0.007	0.001	0.006	<0.001	0.003	0.001	0.004

SEX by Year, Month, Species

		Year=1				Year=2			
		Month=7		Month=1		Month=7		Month=1	
SOURCE	df	*D. mel.*	*D. sim.*	*D. mel.*	*D. sim.*	*D. mel.*	*D. sim.*	*D. mel.*	*D. sim.*
SEX	1	0.103**	0.035**	0.021	0.099**	0.005	0.578**	0.085**	0.026**
error	18	0.010	<0.001	0.005	0.001	0.003	<0.001	0.005	<0.001

**p≤0.01
*p≤0.05
#p=0.06

N.B.: Columnar values are the mean squares.
All scores were corrected using the Or.-R laboratory strain as a yoked control.
All sources were tested over the MS error.

Table 17. Three-factor analyses of variance of vagility of males of *Drosophila* species.

		YEAR, MONTH, SPECIES	
SOURCE	df	4 Hours	24 Hours
YEAR	1	0.007	0.055*
MONTH	1	0.049*	<0.001
SPECIES	1	0.313**	0.017**
YEAR*MONTH	1	0.048*	0.043$^{\#}$
YEAR*SPECIES	1	0.003	0.001
MONTH*SPECIES	1	0.007	<0.001
YEAR*MONTH*SPECIES	1	0.005	0.005
error	72	0.012	0.012

**$p \le 0.01$
*$p \le 0.05$
$^{\#}p=0.06$

N.B.: Columnar values are the mean squares.
All scores were angularly transformed before analysis.
All sources were tested over the MS error.

Table 18. Two-factor analyses of variance of vagility of males of *Drosophila* species.

YEAR, MONTH by Species

		D. melanogaster		*D. simulans*	
SOURCE	df	4 Hours	24 Hours	4 Hours	24 Hours
YEAR	1	0.010	0.021	<0.001	0.034#
MONTH	1	0.046	<0.001	0.010	<0.001
YEAR*MONTH	1	0.042	0.038	0.011	0.010
error	36	0.014	0.014	0.009	0.009

YEAR, SPECIES by Month

		Month=7		Month=1	
SOURCE	df	4 Hours	24 Hours	4 Hours	24 Hours
YEAR	1	0.009	<0.001	0.046*	0.097**
SPECIES	1	0.207**	0.088*	0.113**	0.086**
YEAR*SPECIES	1	<0.001	0.005	0.008	0.001
error	36	0.012	0.015	0.011	0.009

MONTH, SPECIES by Year

		Year=1		Year=2	
SOURCE	df	4 Hours	24 Hours	4 Hours	24 Hours
MONTH	1	<0.001	0.019	0.097**	0.024
SPECIES	1	0.189**	0.076**	0.127**	0.099**
MONTH*SPECIES	1	<0.001	0.002	0.012	0.002
error	36	0.011	0.011	0.012	0.012

**$p \leq 0.01$
*$p \leq 0.05$
#$p = 0.06$

N.B.: Columnar values are the mean squares.
All scores were angularly transformed before analysis.
All sources were tested over the MS error.

Table 19. One-factor analyses of variance of vagility of males of *Drosophila* species.

YEAR by Month, Species

		4 Hours				24 Hours			
		Month=7		Month=1		Month=7		Month=1	
SOURCE	df	*D. mel.*	*D. sim.*	*D. mel.*	*D. sim.*	*D. mel.*	*D. sim.*	*D. mel.*	*D. sim.*
YEAR	1	0.006	0.004	0.046*	0.008	0.001	0.004	0.058**	0.040
error	18	0.019	0.005	0.009	0.013	0.021	0.008	0.007	0.010

MONTH by Year, Species

		4 Hours				24 Hours			
		Year=1		Year=2		Year=1		Year=2	
SOURCE	df	*D. mel.*	*D. sim.*	*D. mel.*	*D. sim.*	*D. mel.*	*D. sim.*	*D. mel.*	*D. sim.*
MONTH	1	<0.001	<0.001	0.088*	0.021	0.017	0.004	0.021	0.005
error	18	0.011	0.012	0.017	0.007	0.008	0.014	0.021	0.004

SPECIES by Year, Month

		4 Hours				24 Hours			
		Year=1		Year=2		Year=1		Year=2	
SOURCE	df	Month=7	Month=1	Month=7	Month=1	Month=7	Month=1	Month=7	Month=1
SPECIES	1	0.099**	0.090*	0.108*	0.031	0.026	0.052*	0.067	0.035*
error	18	0.009	0.014	0.015	0.009	0.011	0.011	0.019	0.006

**$p \leq 0.01$

*$p \leq 0.05$

$^{\#}p = 0.06$

N.B.: Columnar values are the mean squares.
All scores were angularly transformed before analysis.
All sources were tested over the MS error.

Table 20. Four-factor analysis of variance of body size of *Drosophila* species.

	YEAR, MONTH, SPECIES, SEX	
SOURCE	df	MS
YEAR	1	0.009
MONTH	1	0.002
SPECIES	1	1.041**
SEX	1	0.059**
YEAR*MONTH	1	0.185**
YEAR*SPECIES	1	0.002
YEAR*SEX	1	0.031**
MONTH*SPECIES	1	0.017*
MONTH*SEX	1	0.003
SPECIES*SEX	1	0.018*
YEAR*MONTH*SPECIES	1	0.045**
YEAR*MONTH*SEX	1	0.002
YEAR*SPECIES*SEX	1	<0.001
MONTH*SPECIES*SEX	1	0.002
YEAR*MONTH*SPECIES*SEX	1	<0.001
error	144	0.004

**$p \leq 0.01$
*$p \leq 0.05$
$^{\#}p=0.06$

N.B.: Columnar values are the mean squares.
All scores were corrected using the Or.-R laboratory strain as a yoked control.
All sources were tested over the MS error.

Table 21. Three-factor analyses of variance of body size of *Drosophila* species.

		MONTH SPECIES SEX by Year		YEAR SPECIES SEX by Month		YEAR MONTH SEX by Species		YEAR MONTH SPECIES by Sex	
Source	df	Year=1	Year=2	Month=7	Month=1	*D. mel.*	*D. sim.*	male	female
YEAR	1	▨	▨	0.056**	0.137**	0.001	0.010	0.037**	0.004
MONTH	1	0.077**	0.110**	▨	▨	0.004	0.014*	<0.001	0.005
SPECIES	1	0.473**	0.570**	0.398**	0.660**	▨	▨	0.392**	0.668**
SEX	1	0.089**	0.002	0.017*	0.045**	0.006	0.072**	▨	▨
YR*MO	1	▨	▨	▨	▨	0.024*	0.206**	0.074**	0.113**
YR*SP	1	▨	▨	$0.014^{\#}$	0.034**	▨	▨	0.001	0.001
YR*SEX	1	▨	▨	0.025**	0.0009	0.017*	0.015*	▨	▨
MO*SP	1	0.004	0.058**	▨	▨	▨	▨	0.003	0.015*
MO*SEX	1	<0.001	0.005	▨	▨	<0.001	0.0005	▨	▨
SP*SEX	1	0.008	0.010	0.004	0.016*	▨	▨	▨	▨
YR*MO*SP	1	▨	▨	▨	▨	▨	▨	0.017*	0.029**
YR*MO*SEX	1	▨	▨	▨	▨	<0.001	0.003	▨	▨
YR*SP*SEX	1	▨	▨	<0.001	0.001	▨	▨	▨	▨
MO*SP*SEX	1	<0.001	0.003	▨	▨	▨	▨	▨	▨
error	72	0.003	0.005	0.004	0.004	0.004	0.003	0.003	0.004

**p≤0.01
*p≤0.05
$^{\#}p=0.06$

N.B.: Columnar values are the mean squares.
Shading indicates sources not possible for various factor combinations.
All scores were corrected using the Or.-R laboratory strain as a yoked control.
All sources were tested over the MS error.

Table 22. Two-factor analyses of variance of body size of *Drosophila* species.

YEAR, MONTH by Species, Sex

		D. melanogaster		*D. simulans*	
SOURCE	df	male	female	male	female
YEAR	1	0.013#	0.005	0.025**	<0.001
MONTH	1	0.003	0.002	0.001	0.018*
YEAR*MONTH	1	0.010	0.014	0.081**	0.128**
error	36	0.003	0.005	0.003	0.003

YEAR, SPECIES by Month, Sex

		Month=7		Month-1	
SOURCE	df	male	female	male	female
YEAR	1	0.003	0.078**	0.107**	0.038**
SPECIES	1	0.161**	0.240**	0.234**	0.443**
YEAR*SPECIES	1	0.005	0.009	0.013*	0.022*
error	36	0.004	0.004	0.003	0.004

YEAR, SEX by Month, Species

		Month=7		Month-1	
SOURCE	df	*D. mel.*	*D. sim.*	*D. mel.*	*D. sim.*
YEAR	1	0.007	0.063**	0.017*	0.154**
SEX	1	0.002	0.019*	0.004	0.058**
YEAR*SEX	1	0.010	0.015*	0.007	0.002
error	36	0.004	0.003	0.004	0.003

Table 22. continued.

MONTH, SPECIES by Year, Sex

		Year=1		Year=2	
SOURCE	df	male	female	male	female
MONTH	1	0.040**	0.036**	0.034**	0.081**
SPECIES	1	0.178**	0.304**	0.215**	0.365**
MONTH*SPECIES	1	0.003	0.001	0.018*	0.043**
error	36	0.002	0.003	0.004	0.005

MONTH, SEX by Year, Species

		YEAR=1		YEAR=2	
SOURCE	df	*D. mel.*	*D. sim.*	*D. mel.*	*D. sim.*
MONTH	1	0.024**	0.056**	0.004	0.164**
SEX	1	0.021**	0.076**	0.001	0.011
MONTH*SEX	1	<0.001	<0.001	<0.001	0.008
error	36	0.003	0.003	0.005	0.004

SPECIES, SEX by Year, Month

		Year=1		Year=2	
SOURCE	df	Month=7	Month=1	Month=7	Month=1
SPECIES	1	0.279**	0.198**	0.132**	0.496**
SEX	1	0.042**	0.047**	<0.001	0.007
SPECIES*SEX	1	0.003	0.005	0.001	0.012
error	36	0.002	0.003	0.005	0.004

$^{**}p \leq 0.01$
$^{*}p \leq 0.05$
$^{\#}p = 0.06$

N.B.: Columnar values are the mean squares.
All scores were corrected using the Or.-R laboratory strain as a yoked control.
All sources were tested over the MS error.

Table 23. One-factor analyses of variance of body size of *Drosophila* species.

YEAR by Month, Species, Sex

		Month=7				Month=1			
		D. melanogaster		*D. simulans*		*D. melanogaster*		*D. simulans*	
SOURCE	df	male	female	male	female	male	female	male	female
YEAR	1	<0.001	0.017#	0.008	0.070**	0.023*	0.001	0.097**	0.059**
error	18	0.003	0.004	0.004	0.003	0.004	0.005	0.003	0.004

MONTH by Year, Species, Sex

		Year=1				Year=2			
		D. melanogaster		*D. simulans*		*D. melanogaster*		*D. simulans*	
SOURCE	df	male	female	male	female	male	female	male	female
MONTH	1	0.011*	0.012#	0.032**	0.025*	0.001	0.003	0.050**	0.122**
error	18	0.003	0.003	0.002	0.003	0.004	0.006	0.004	0.003

SPECIES by Year, Month, Sex

		Year=1				Year=2			
		Month=7		Month=1		Month=7		Month=1	
SOURCE	df	male	female	male	female	male	female	male	female
SPECIES	1	0.112**	0.171**	0.069**	0.134**	0.055**	0.079**	0.178**	0.330**
error	18	0.002	0.002	0.003	0.004	0.005	0.005	0.004	0.004

SEX by Year, Month, Species

		Year=1				Year=2			
		Month=7		Month=1		Month=7		Month=1	
SOURCE	df	*D. mel.*	*D. sim.*	*D. mel.*	*D. sim.*	*D. mel.*	*D. sim.*	*D. mel.*	*D. sim.*
SEX	1	0.011*	0.034**	0.010	0.042**	0.001	<0.001	<0.001	0.018*
error	18	0.002	0.002	0.003	0.003	0.06	0.004	0.005	0.003

**p≤0.01
*p≤0.05
#p=0.06

N.B.: Columnar values are the mean squares.
All scores were corrected using the Or.-R laboratory strain as a yoked control.
All sources were tested over the MS error.

Table 24. Monte Carlo estimates of desiccation resistance proportions of males of *Drosophila* species.

		MALES					
		D. melanogaster			*D. simulans*		
Collection / Hrs		LCL	$\hat{S}_A^2$	UCL	LCL	$\hat{S}_A^2$	UCL
8807	4	-0.0002	0.0002	0.0008	-0.0031	<0.0000	0.0042
	8	-0.0023	0.0000	0.0032	-0.0077	<0.0000	0.0107
	12	-0.0053	<0.0000	0.0074	-0.0072	0.0000	0.0100
	24	-0.0042	0.0005	0.0072	0.0000	0.0000	0.0000
8901	4	-0.0001	<0.0000	0.0002	-0.0022	0.0000	0.0030
	8	-0.0002	0.0001	0.0005	-0.0025	0.0107*	0.0310
	12	-0.0034	0.0000	0.0048	0.0013	0.0133**	0.0325
	24	-0.0007	0.0004	0.0020	-0.0000	<0.0000	0.0000
8907	4	<0.0000	0.0003**	0.0008	-0.0005	0.0005	0.0019
	8	-0.0010	0.0015	0.0052	-0.0037	0.0068	0.0226
	12	-0.0031	0.0016	0.0086	-0.0102	-0.0002	0.0138
	24	-0.0009	<0.0000	0.0012	0.0000	0.0000	0.0000
9001	4	<0.0000	0.0000	0.0000	-0.0013	0.0010	0.0045
	8	-0.0003	0.0021*	0.0058	-0.0123	0.0000	0.0172
	12	-0.0034	0.0047	0.0171	-0.0150	-0.0001	0.0205
	24	-0.0008	0.0002	0.0016	0.0000	0.0000	0.0000

$^{**}p \leq 0.01$
$^{*}p \leq 0.05$
$^{\#}p = 0.06$

LCL = Lower Confidence Limit
UCL = Upper Confidence Limit

Table 25. Monte Carlo estimates of desiccation resistance proportions of females of *Drosophila* species.

		FEMALES					
		D. melanogaster			*D. simulans*		
Collection	Hrs	LCL	$\hat{S}_A^2$	UCL	LCL	$\hat{S}_A^2$	UCL
8807	4	-0.0001	$0.0003^{\#}$	0.0010	-0.0006	0.0000	0.0008
	8	-0.0008	<0.0000	0.0011	-0.0042	<0.0000	0.0059
	12	-0.0025	0.0000	0.0035	-0.0096	<0.0000	0.0133
	24	-0.0081	0.0030	0.0191	<0.0000	$0.0002^{\#}$	0.0005
8901	4	<0.0000	<0.0000	0.0000	-0.0003	0.0000	0.0005
	8	<0.0000	0.0000	0.0000	-0.0002	0.0057**	0.0150
	12	-0.0006	0.0004	0.0019	-0.0090	0.0032	0.0209
	24	-0.0050	0.0023	0.0130	-0.0015	0.0005	0.0033
8907	4	<0.0000	0.0000	0.0003	<0.0000	<0.0000	0.0001
	8	-0.0005	0.0008	0.0029	-0.0017	0.0009	0.0048
	12	-0.0018	0.0030	0.0103	-0.0049	0.0114*	0.0367
	24	-0.0101	0.0000	0.0142	<0.0000	<0.0000	0.0000
9001	4	<0.0000	<0.0000	0.0000	-0.0007	0.0004	0.0021
	8	-0.0003	-0.0002	0.0010	-0.0082	0.0044	0.0231
	12	-0.0022	<0.0000	0.0031	-0.0115	0.0020	0.0215
	24	-0.0081	0.0000	0.0112	<0.0000	0.0000	0.0000

$^{**}p \leq 0.01$
$^{*}p \leq 0.05$
$^{\#}p = 0.06$

LCL = Lower Confidence Limit
UCL = Upper Confidence Limit

Table 26. Monte Carlo estimates of vagility scores of *Drosophila* species.

		MALES (only)					
		D. melanogaster			*D. simulans*		
Collection / Hrs		LCL	$\hat{S}^2_A$	UCL	LCL	$\hat{S}^2_A$	UCL
8807	4	-0.0020	0.0058*	0.0174	-0.0038	0.0000	0.0047
	24	-0.0027	0.0040	0.0139	-0.0041	0.0033	0.0136
8901	4	-0.0039	0.0017	0.0092	0.0014	0.0093**	0.0218
	24	-0.0027	0.0013	0.0067	0.0011	0.0120**	0.0290
8907	4	-0.0065	0.0073	0.0266	-0.0071	0.0000	0.0087
	24	0.0010	0.0171**	0.0424	-0.0069	0.0000	0.0083
9001	4	-0.0036	0.0016	0.0086	-0.0023	0.0012	0.0060
	24	-0.0026	0.0030	0.0108	-0.0034	0.0000	0.0041

**$p \leq 0.01$
*$p \leq 0.05$
$^{\#}p=0.06$

LCL = Lower Confidence Limit
UCL = Upper Confidence Limit

Table 27. Monte Carlo estimates of body size measurements of *Drosophila* species.

MALES

Collection	*D. melanogaster* LCL	*D. melanogaster* $\hat{S}^2_A$	*D. melanogaster* UCL	*D. simulans* LCL	*D. simulans* $\hat{S}^2_A$	*D. simulans* UCL
8807	0.0000	0.0013**	0.0033	-0.0004	0.0014*	0.0040
8901	0.0003	0.0026**	0.0062	0.0000	0.0014**	0.0034
8907	0.0006	0.0040**	0.0094	0.0006	0.0045**	0.0106
9001	0.0000	0.0029**	0.0075	0.0003	0.0027**	0.0066

FEMALES

Collection	*D. melanogaster* LCL	*D. melanogaster* $\hat{S}^2_A$	*D. melanogaster* UCL	*D. simulans* LCL	*D. simulans* $\hat{S}^2_A$	*D. simulans* UCL
8807	-0.0003	0.0014*	0.0041	-0.0008	0.0007	0.0028
8901	0.0000	0.0026**	0.0065	0.0009	0.0045**	0.0102
8907	0.0012	0.0057**	0.0128	0.0002	0.0026**	0.0063
9001	0.0000	0.0046**	0.0116	0.0000	0.0015**	0.0038

**$p \leq 0.01$
*$p \leq 0.05$
#$p = 0.06$

LCL = Lower Confidence Limit
UCL = Upper Confidence Limit

Table 28. Correlation analyses by Species and Sex of *Drosophila* species.

MALES

		Drosophila melanogaster					*Drosophila simulans*				
		Des. Res. 4 hours	Des. Res. 8 hours	Des. Res. 12 hours	Des. Res. 24 hours	Body Size	Des. Res. 4 hours	Des. Res. 8 hours	Des. Res. 12 hours	Des. Res. 24 hours	Body Size
Vag.	r	-0.0545	-0.1863	-0.0484	-0.1806	0.0735	-0.0352	-0.2379	-0.1724	-0.2196	-0.0885
4 hrs	p	0.7382	0.2498	0.7666	0.2649	0.6521	0.8292	0.1394	0.2875	0.1733	0.5872
	r^2	0.0030	0.0347	0.0023	0.0326	0.0054	0.0012	0.0566	0.0297	0.0482	0.0078
Vag.	r	0.1609	0.0523	-0.0866	-0.1492	0.0608	-0.1591	-0.4199	-0.2531	-0.1851	0.1161
24 hrs	p	0.3214	0.7487	0.5851	0.3581	0.7094	0.3267	0.0070**	0.1151	0.2527	0.4871
	r^2	0.0259	0.0027	0.0075	0.0223	0.0037	0.0253	0.1763	0.0641	0.0343	0.0128
Body	r	-0.2952	-0.2426	-0.2392	-0.0131	▨	-0.0303	-0.2401	-0.5162	-0.6265	▨
Size	p	0.0644#	0.1315	0.1371	0.9358	▨	0.8526	0.1356	0.0007**	0.0001**	▨
	r^2	0.0871	0.0589	0.0572	0.0002	▨	0.0009	0.0576	0.2665	0.3925	▨

FEMALES

		Drosophila melanogaster					*Drosophila simulans*				
		Des. Res. 4 hours	Des. Res. 8 hours	Des. Res. 12 hours	Des. Res. 24 hours	Body Size	Des. Res. 4 hours	Des. Res. 8 hours	Des. Res. 12 hours	Des. Res. 24 hours	Body Size
Body	r	-0.1065	-0.1979	-0.4731	-0.1740	▨	0.2133	0.1832	-0.3353	-0.6814	▨
Size	p	0.5131	0.2208	0.0021**	0.2830	▨	0.1863	0.2578	0.0344*	0.0001**	▨
	r^2	0.0113	0.0392	0.2238	0.0303	▨	0.0455	0.0336	0.1124	0.4643	▨

$^{**}p \leq 0.01$
$^{*}p \leq 0.05$
$^{\#}p = 0.06$

N.B.: Shading indicates analyses not possible.

Table 29. Correlation analyses by Year, Species, and Sex of *Drosophila* species..

YEAR=1

MALES

		Drosophila melanogaster					*Drosophila simulans*				
		Des. Res. 4 hours	Des. Res. 8 hours	Des. Res. 12 hours	Des. Res. 24 hours	Body Size	Des. Res. 4 hours	Des. Res. 8 hours	Des. Res. 12 hours	Des. Res. 24 hours	Body Size
Vag.	r	-0.2988	-0.5780	-0.0144	-0.0318	0.2564	-0.2674	-0.5943	-0.1594	-0.0580	0.0824
4 hrs	p	0.2007	0.0076**	0.9520	0.8940	0.2752	0.2545	0.0057**	0.5020	0.8081	0.7297
	r^2	0.0893	0.3341	0.0002	0.0010	0.0657	0.0715	0.3532	0.0254	0.0034	0.0068
Vag.	r	-0.0049	-0.1851	-0.2792	0.0110	0.3749	-0.0408	-0.5322	-0.2545	-0.1248	0.0062
24 hrs	p	0.9836	0.4347	0.2333	0.4663	0.1034	0.8644	0.0157*	0.2789	0.6002	0.9793
	r^2	0.0000	0.0343	0.0780	0.0001	0.1406	0.0017	0.2832	0.0648	0.0156	0.0000
Body	r	0.0378	-0.0522	-0.4067	-0.1728		0.2221	-0.4379	-0.6573	-0.6618	
Size	p	0.8743	0.8271	0.0751	0.4663		0.3467	0.0535*	0.0016**	0.0015**	
	r^2	0.0014	0.0027	0.1654	0.0299		0.0493	0.1918	0.4320	0.4380	

YEAR=1

FEMALES

		Drosophila melanogaster					*Drosophila simulans*				
		Des. Res. 4 hours	Des. Res. 8 hours	Des. Res. 12 hours	Des. Res. 24 hours	Body Size	Des. Res. 4 hours	Des. Res. 8 hours	Des. Res. 12 hours	Des. Res. 24 hours	Body Size
Body	r	0.2057	0.1273	-0.4144	-0.2120		0.0055	-0.0255	-0.2622	-0.3984	
Size	p	0.3843	0.5927	0.0693	0.3696		0.9817	0.9151	0.2640	0.0819	
	r^2	0.0423	0.0162	0.1717	0.0449		0.0000	0.0007	0.0687	0.1587	

Table 29. Continued.

YEAR=2
MALES

		Drosophila melanogaster					*Drosophila simulans*				
		Des. Res. 4 hours	Des. Res. 8 hours	Des. Res. 12 hours	Des. Res. 24 hours	Body Size	Des. Res. 4 hours	Des. Res. 8 hours	Des. Res. 12 hours	Des. Res. 24 hours	Body Size
Vag.	r	0.2153	0.0330	-0.1404	-0.3648	-0.0912	0.2998	0.2282	-0.2347	-0.3709	0.0853
4 hrs	p	0.3620	0.8903	0.5550	0.1138	0.7021	0.1990	0.3332	0.3193	0.1074	0.7207
	r^2	0.0454	0.0011	0.0197	0.1331	0.0083	0.0899	0.0521	0.0551	0.1376	0.0073
Vag.	r	0.4329	0.2339	0.0499	-0.3057	-0.1875	0.0862	-0.0736	-0.3331	-0.2626	0.0288
24 hrs	p	0.0566#	0.3210	0.8346	0.1900	0.4283	0.7180	0.7577	0.1513	0.2634	0.9041
	r^2	0.1874	0.0547	0.0025	0.0935	0.0352	0.0074	0.0054	0.1110	0.0690	0.0008
Body	r	-0.4662	-0.2372	-0.1504	0.2596	▨	0.1535	0.0427	-0.5971	-0.6203	▨
Size	p	0.0383*	0.3139	0.5267	0.2690	▨	0.5181	0.8583	0.0054**	0.0035**	▨
	r^2	0.2173	0.0563	0.0226	0.0674	▨	0.0236	0.0018	0.3565	0.3848	▨

YEAR=2
FEMALES

		Drosophila melanogaster					*Drosophila simulans*				
		Des. Res. 4 hours	Des. Res. 8 hours	Des. Res. 12 hours	Des. Res. 24 hours	Body Size	Des. Res. 4 hours	Des. Res. 8 hours	Des. Res. 12 hours	Des. Res. 24 hours	Body Size
Body	r	-0.6666	-0.4409	-0.5054	0.1100	▨	0.3353	0.3049	-0.3846	-0.8387	▨
Size	p	0.0013**	0.0517*	0.0230*	0.6444	▨	0.1484	0.1911	0.0940	0.0001**	▨
	r^2	0.4444	0.1944	0.2554	0.0121	▨	0.1124	0.0930	0.1479	0.7034	▨

**$p \leq 0.01$
*$p \leq 0.05$
#$p = 0.06$

N.B.: Shading indicates analyses not possible.

Table 30. Correlation analyses by MONTH, SPECIES, and SEX of *Drosophila* species.

MONTH=7
MALES

		Drosophila melanogaster					*Drosophila simulans*				
		Des. Res. 4 hours	Des. Res. 8 hours	Des. Res. 12 hours	Des. Res. 24 hours	Body Size	Des. Res. 4 hours	Des. Res. 8 hours	Des. Res. 12 hours	Des. Res. 24 hours	Body Size
Vag.	r	0.2741	0.0772	-0.0141	-0.0882	0.0018	-0.0218	-0.1179	-0.1930	-0.1916	0.1214
4 hrs	p	0.2422	0.7463	0.9530	0.7117	0.9940	0.9272	0.6206	0.4149	0.4184	0.6101
	r^2	0.0751	0.0060	0.0002	0.0078	0.0000	0.0005	0.0139	0.0372	0.0367	0.0147
Vag.	r	0.3999	0.2875	0.1201	-0.0419	-0.0941	-0.2493	-0.3836	0.0127	0.1623	-0.2428
24 hrs	p	0.0807	0.2191	0.6142	0.8607	0.6931	0.2893	0.0950	0.9575	0.4942	0.3024
	r^2	0.1599	0.0827	0.0144	0.0018	0.0089	0.0622	0.1471	0.0002	0.0263	0.0590
Body	r	-0.5148	-0.2868	0.0038	0.1943		0.1259	-0.1028	-0.2854	-0.3214	
Size	p	0.0202*	0.2203	0.9874	0.4118		0.5968	0.6662	0.2225	0.1671	
	r^2	0.2650	0.0823	0.0000	0.0378		0.0159	0.0106	0.0815	0.1033	

MONTH=7
FEMALES

		Drosophila melanogaster					*Drosophila simulans*				
		Des. Res. 4 hours	Des. Res. 8 hours	Des. Res. 12 hours	Des. Res. 24 hours	Body Size	Des. Res. 4 hours	Des. Res. 8 hours	Des. Res. 12 hours	Des. Res. 24 hours	Body Size
Body	r	-0.1918	-0.5083	-0.6347	-0.2218		0.2071	0.1102	-0.7023	-0.7690	
Size	p	0.4178	0.0221*	0.0026**	0.3474		0.3809	0.6436	0.0006**	0.0001**	
	r^2	0.0368	0.2584	0.4028	0.0492		0.0429	0.0121	0.4932	0.5914	

Table 30. Continued.

MONTH=1
MALES

		Drosophila melanogaster					*Drosophila simulans*				
		Des. Res. 4 hours	Des. Res. 8 hours	Des. Res. 12 hours	Des. Res. 24 hours	Body Size	Des. Res. 4 hours	Des. Res. 8 hours	Des. Res. 12 hours	Des. Res. 24 hours	Body Size
Vag. 4 hrs	r	-0.4951	-0.3732	-0.4180	-0.3900	0.2407	-0.0548	-0.4755	-0.3115	-0.2104	0.0591
	p	0.0264*	0.1051	0.0666	0.0892	0.3066	0.8186	0.0341*	0.1812	0.3732	0.8045
	r^2	0.2451	0.1393	0.1747	0.1521	0.0579	0.0030	0.2261	0.0970	0.0443	0.0035
Vag. 24 hrs	r	-0.4708	-0.3070	-0.4624	-0.3393	0.2527	-0.0738	-0.5330	-0.5079	-0.4148	0.3216
	p	0.0361*	0.1880	0.0401*	0.1434	0.2824	0.7571	0.0155*	0.0222*	0.0690	0.1660
	r^2	0.2217	0.0942	0.2138	0.1151	0.0639	0.0054	0.2841	0.2580	0.1721	0.1036
Body Size	r	-0.2780	-0.3284	-0.4126	-0.2044	▨	-0.1907	-0.4241	-0.8211	-0.8111	▨
	p	0.2353	0.1575	0.0706	0.3874	▨	0.4206	0.0624$^{\#}$	0.0001**	0.0001**	▨
	r^2	0.0773	0.1078	0.1702	0.0418	▨	0.0364	0.1799	0.6742	0.6579	▨

MONTH=1
FEMALES

		Drosophila melanogaster					*Drosophila simulans*				
		Des. Res. 4 hours	Des. Res. 8 hours	Des. Res. 12 hours	Des. Res. 24 hours	Body Size	Des. Res. 4 hours	Des. Res. 8 hours	Des. Res. 12 hours	Des. Res. 24 hours	Body Size
Body Size	r	-0.1397	-0.0303	-0.2262	-0.1458	▨	0.2143	0.2189	-0.0436	-0.5590	▨
	p	0.5568	0.8989	0.3375	0.5398	▨	0.3643	0.3538	0.8557	0.0104*	▨
	r^2	0.0195	0.0009	0.0512	0.0213	▨	0.0459	0.0479	0.0019	0.3125	▨

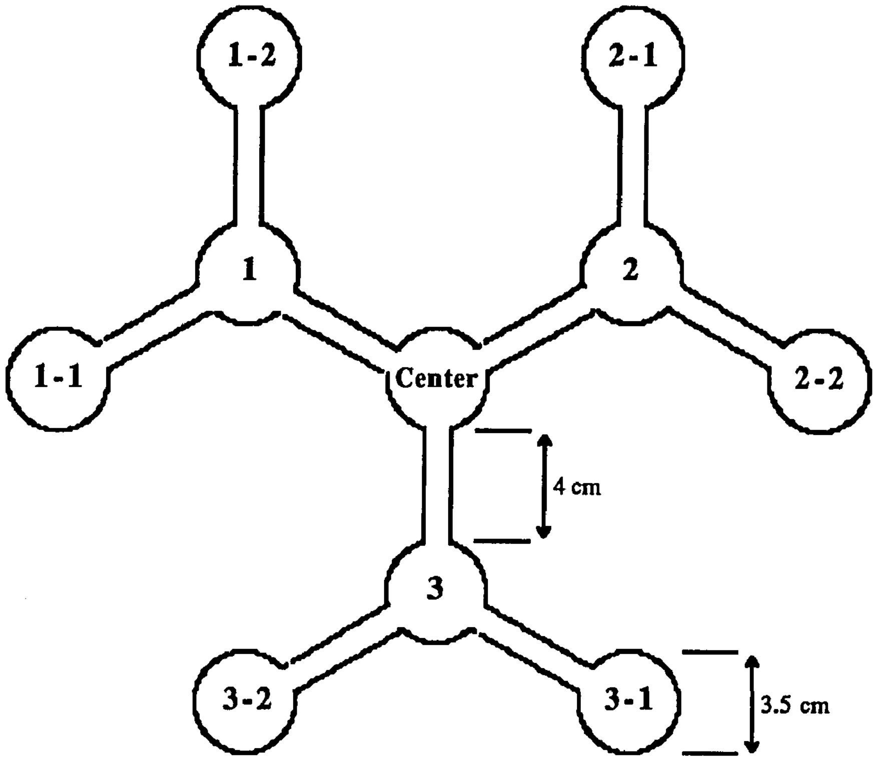
$^{**}p \leq 0.01$
$^{*}p \leq 0.05$
$^{\#}p = 0.06$

N.B.: Shading indicates analyses not possible.

Figure 1. Location of Laguna Verde, site of the collections of *Drosophila melanogaster* and *D. simulans*.

Figure 2. Adult double-ringed vagility test system.

(A) Adult double-ringed vagility test system showing dimensions.



(B) Single vial showing connecting tubes and giving various dimensions.

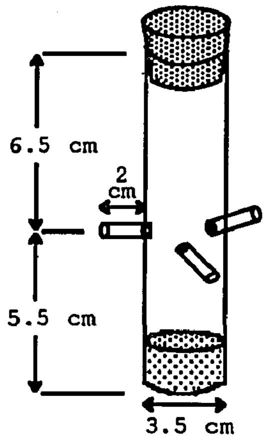


Figure 3. Wing of *Drosophila*.
The two boundaries used for the measurement of wing length using the III Longitudinal vein.

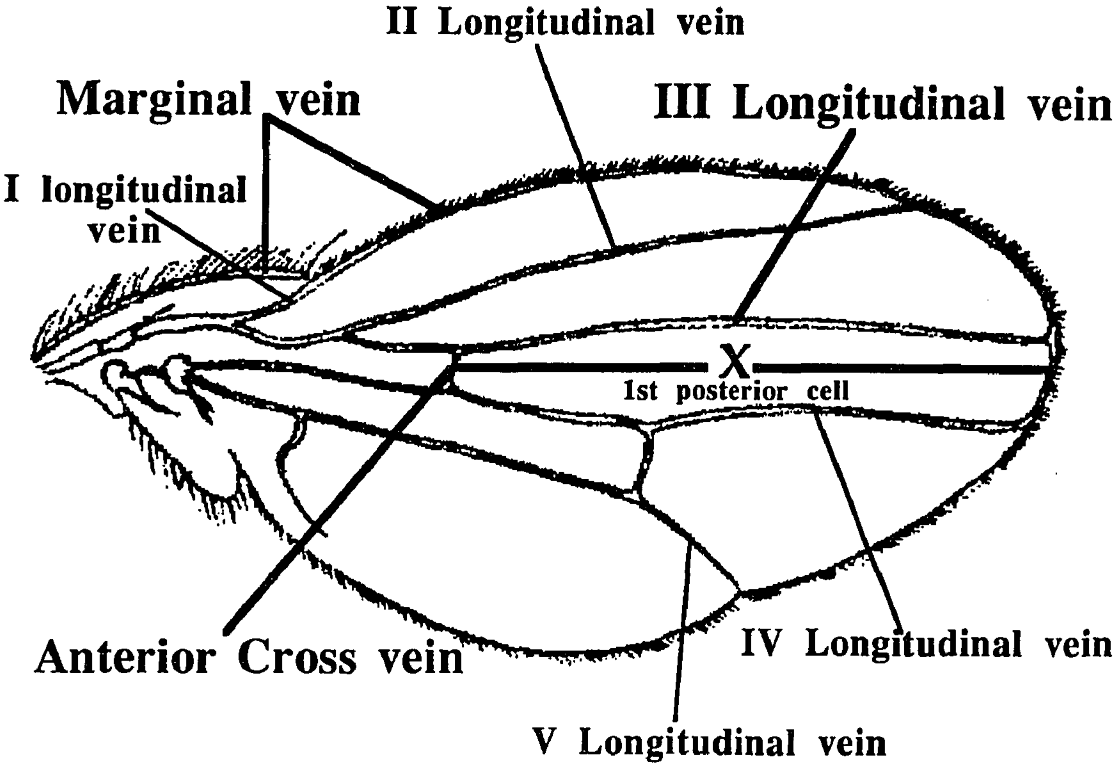
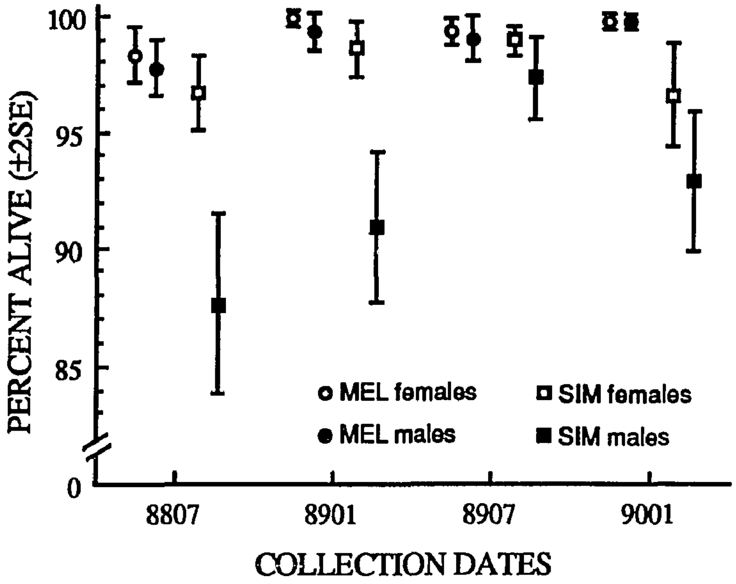
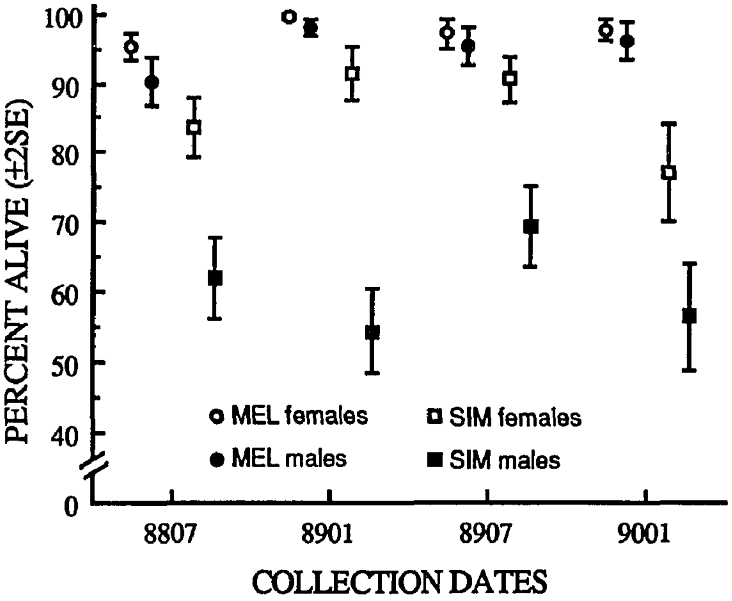


Figure 4. Graph of mean percent survival after 4 hrs, at 0% RH and 20°C, of flies collected at various dates. MEL, *D. melanogaster*; SIM, *D. simulans*



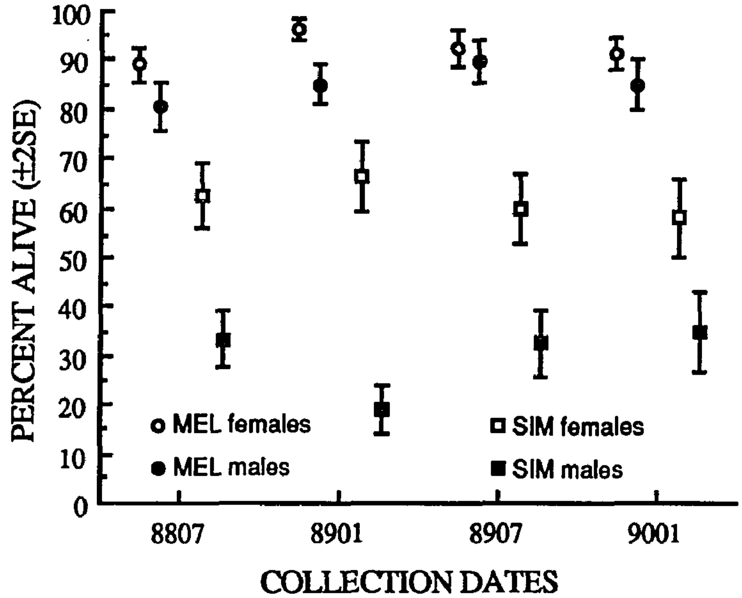
N.B.: The data for this figure has been published in Rockwell, R.F., M.E. de la Rosa, J. Guzmán, M.J. Laverde, L. Levine, and O. Olvera. 1991. A temporal study of desiccation resistance of sibling *Drosophila* species from Laguna Verde, Veracruz, Mexico. *American Midland Naturalist* **126**(2):338-344.

Figure 5. Graph of mean percent survival after 8 hrs, at 0% RH and 20°C, of flies collected at various dates. MEL, *D. melanogaster*; SIM, *D. simulans*



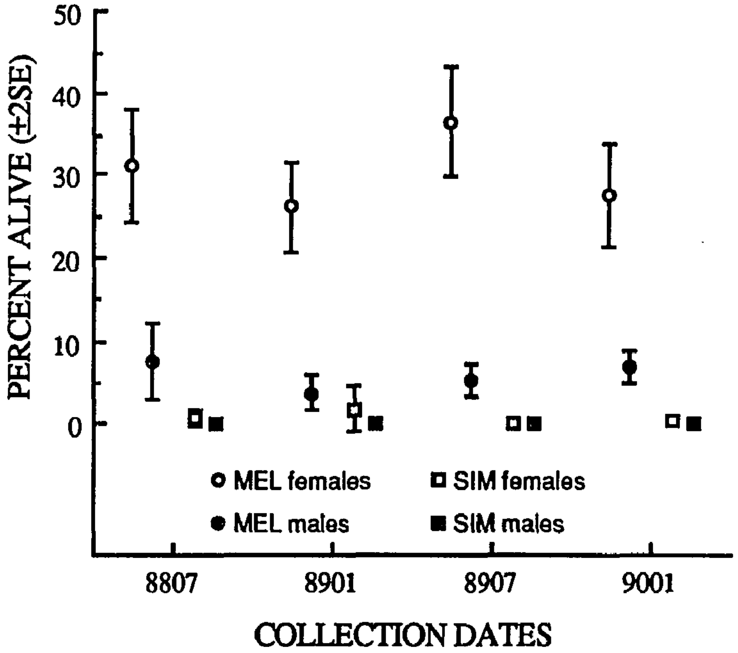
N.B.: The data for this figure has been published in Rockwell, R.F., M.E. de la Rosa, J. Guzmán, M.J. Laverde, L. Levine, and O. Olvera. 1991. A temporal study of desiccation resistance of sibling *Drosophila* species from Laguna Verde, Veracruz, Mexico. *American Midland Naturalist* **126**(2):338-344.

Figure 6. Graph of mean percent survival after 12 hrs, at 0% RH and 20°C, of flies collected at various dates. MEL, *D. melanogaster*; SIM, *D. simulans*



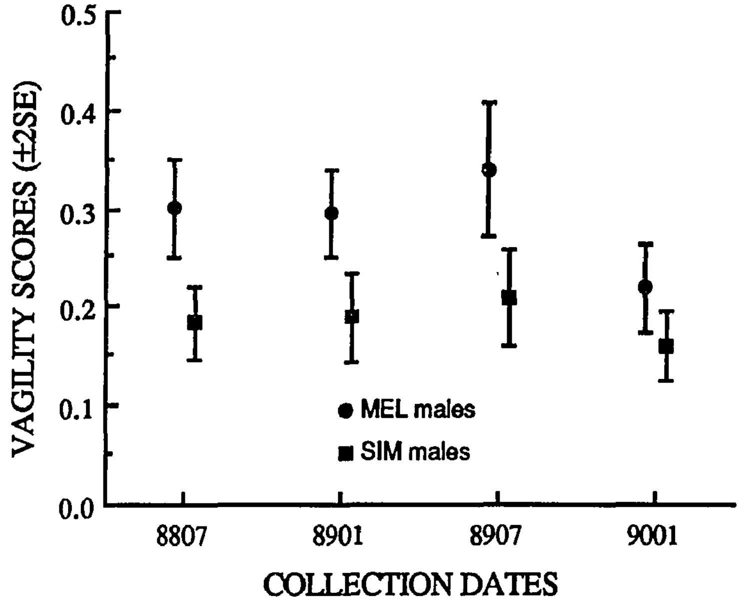
N.B.: The data for this figure has been published in Rockwell, R.F., M.E. de la Rosa, J. Guzmán, M.J. Laverde, L. Levine, and O. Olvera. 1991. A temporal study of desiccation resistance of sibling *Drosophila* species from Laguna Verde, Veracruz, Mexico. *American Midland Naturalist* **126**(2):338-344.

Figure 7. Graph of mean percent survival after 24 hrs, at 0% RH and 20°C, of flies collected at various dates. MEL, *D. melanogaster*; SIM, *D. simulans*



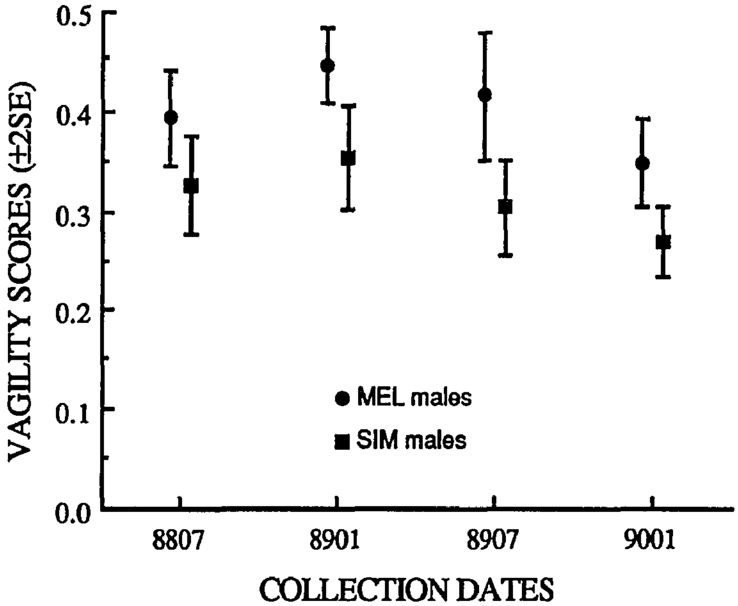
N.B.: The data for this figure has been published in Rockwell, R.F., M.E. de la Rosa, J. Guzmán, M.J. Laverde, L. Levine, and O. Olvera. 1991. A temporal study of desiccation resistance of sibling *Drosophila* species from Laguna Verde, Veracruz, Mexico. *American Midland Naturalist* **126**(2):338-344.

Figure 8. Graph of mean vagility scores of males at 4 hrs of flies collected at various dates. MEL, *D. melanogaster*; SIM, *D. simulans*



N.B.: The data for this figure has been published in Olvera, O., R.F. Rockwell, M.E. de la Rosa, J. Guzmán, M.J. Laverde, and L. Levine. 1993. Studies of Sibling *Drosophila* species from Laguna Verde, Veracruz, Mexico: V. Temporal Stability in Dispersive Behavior. *Southwestern Naturalist* 38(1):15-18.

Figure 9. Graph of mean vagility scores of males at 24 hrs of flies collected at various dates. MEL, *D. melanogaster*; SIM, *D. simulans*



N.B.: The data for this figure has been published in Olvera, O., R.F. Rockwell, M.E. de la Rosa, J. Guzmán, M.J. Laverde, and L. Levine. 1993. Studies of Sibling *Drosophila* species from Laguna Verde, Veracruz, Mexico: V. Temporal Stability in Dispersive Behavior. *Southwestern Naturalist* 38(1):15-18.

Figure 10. Graph of mean body size (mm) of flies collected at various dates. MEL, *D. melanogaster*; SIM, *D. simulans*

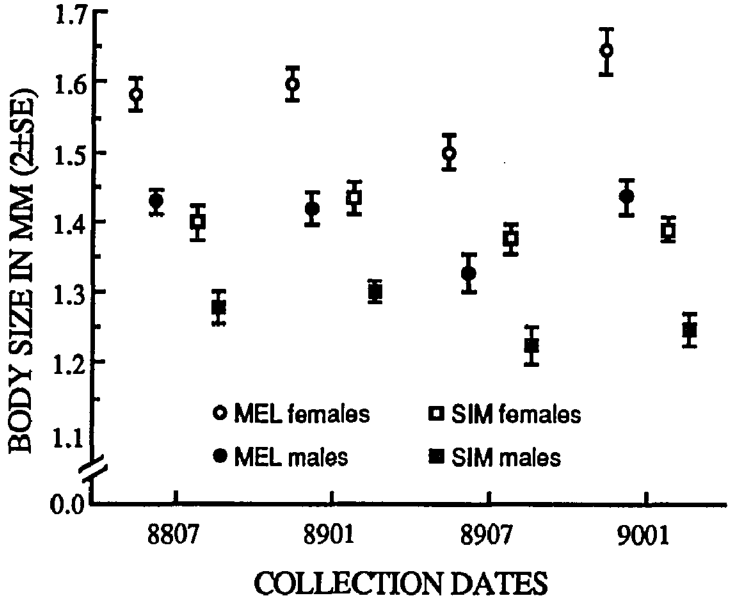
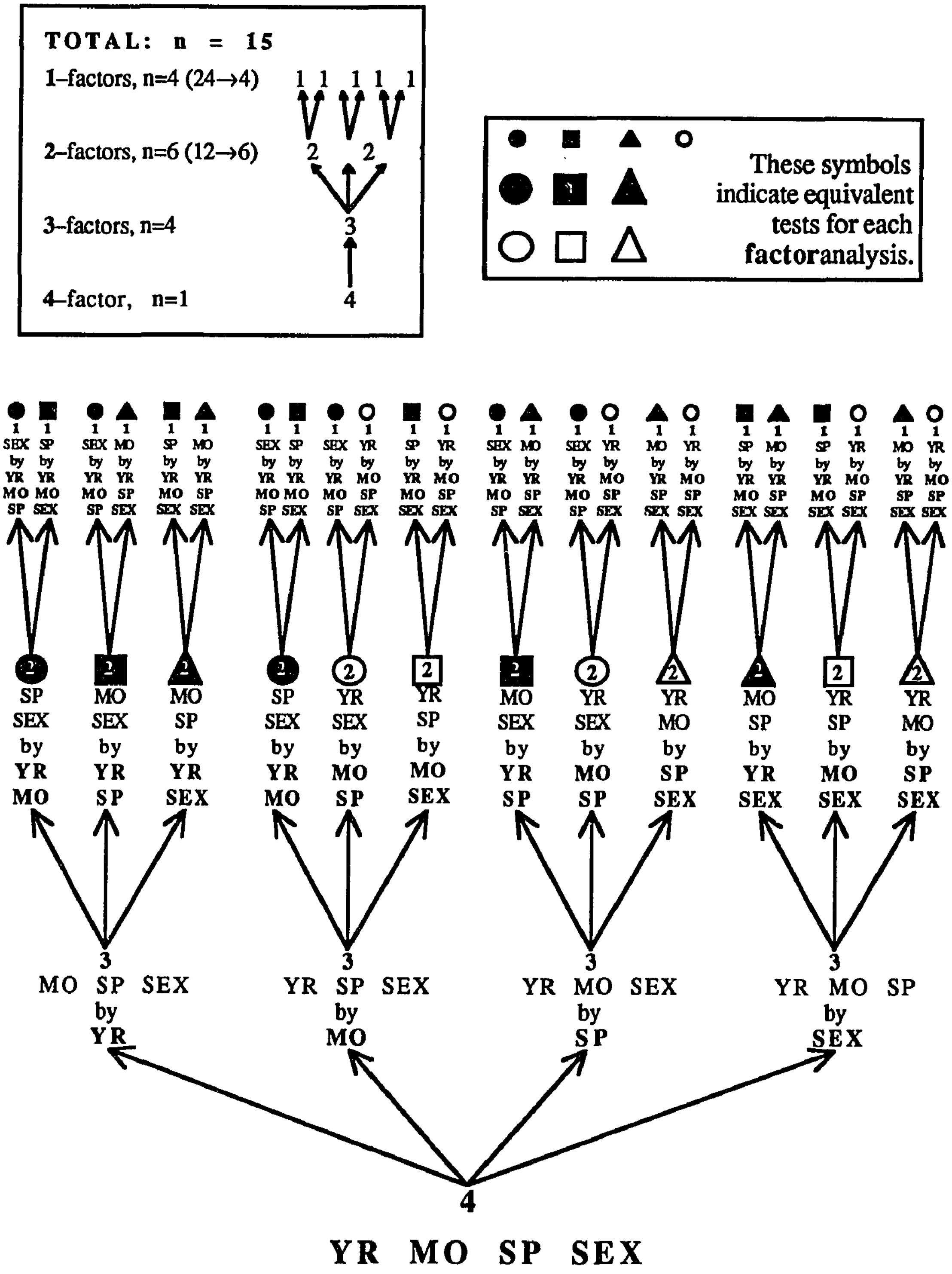


Figure 11. Schematic example of hierarchical analyses of variance approach. YR, year; MO, month; SP, species; SEX, sex.



DISCUSSION

Desiccation resistance, vagility, and body size may all be involved in allowing individual *Drosophila* to survive the rigors of a hostile, desiccating environment. If there is sufficient phenotypic latitude or genetic variance, one might expect short-term changes in the traits to be concordant. If the environmental factors leading to these changes are general, then one might also expect changes to be concordant across two closely related sibling species like *Drosophila melanogaster* and *D. simulans*. As part of the examination of these issues, I have compared the mean responses for the traits both within and between the species for four samples spanning two years and two seasons.

Factorial analyses of variance (ANOVAs) first allow the assessment of whether the factors of year, season, species, and sex affect the traits of desiccation resistance, vagility, and body size independently. Univariate ANOVAs tested the traits for what differences and trends exist in the data for these factors, and whether the general trends are the same for each trait.

Generally for desiccation resistance, the factors of year, season, species, and sex are interdependent. All four factors have been shown to be functionally related in all combinations except the combination of season, species, and sex. The most notable interdependencies are: year, season, and sex; year and season; season and sex; and, species and sex.

Significant differences between the species exist. At all time intervals, *D. melanogaster* is superior to *D. simulans* at resisting desiccation. Stanley *et al.* (1980) found similar results for flies collected from Melbourne, Australia. They also found the desiccation survival of *D. melanogaster* was not affected over a temperature range; but was for *D. simulans*. According to Parsons (1979), *D. melanogaster* tended to replace *D. simulans* when temperature fluctuations became extreme.

Significant temporal differences for desiccation resistance between male and female flies of the same species were found. At resisting desiccation, females were superior to males of *D. simulans* at four and eight hours; and for both species at twelve and twenty four hours. This is the same as found by Rosa *et al.* (1989) and Guzmán *et al.* (1989). Levine *et al.* (1986) found that females had a greater desiccation resistance than males in *D. pseudoobscura*. Ringo and Wood (1984) also found that females had greater desiccation resistance than males, and that this decreased with age. *D. simulans* probably showed earlier sex differences because its has a lower survival rate (faster death rate) than *D. melanogaster* in the desiccator.

There is no clear indication of annual or seasonal variation or cycling in desiccation resistance for males or females from either species. McKenzie and Parsons (1974) also found no seasonal variation for these sibling species collected from Brisbane, Australia, and for *D. melanogaster* from Melbourne, Australia. They did, however, find seasonal variation showing greater resistance during the warmest months of the year for *D. simulans* from Melbourne. These flies may have sufficient genetic variation in the population to vary between greater and lesser resistance at different seasons. McKenzie and Parsons' other collections and the Laguna Verde collections may have followed different adaptive paths; or the latter possibly lacks the genetic variation to change during the year. Possibly, these populations are at their genetic extremes for resisting desiccation displaying no genetic variation for such seasonal cycling; or use other traits along with desiccation resistance like vagility.

For vagility, the traits of year, season, and species are independent or not functionally related. There is no clear indication of annual or seasonal change for vagility (males) for the Laguna Verde populations of these *Drosophila*. However, Levine *et al.* (1989) found a significant seasonal difference for *D. melanogaster* and *D. simulans*. *D. melanogaster* always shows a greater (usually significantly so) vagility than *D. simulans*. Rosa *et al.* (1989) also tested these two sibling species for vagility and found the same results. Both species have a higher vagility score over time for the long-term reading. Given more time, each species' dispersal is increased. If there is a maximum dispersal, it is not reached in the short-term. But *D. melanogaster* still remains more vagile than its sibling species. Vagility may also be used with fitness traits such as body size to better improve survival of these species in a desiccating environment.

For body size, the factors of year, season, species, and sex are interdependent. Year with month and sex (as well as these three factors together), and species with month and sex are functionally related. Regardless of the functional relatedness of these factors, some generalizations are possible.

Body size shows no clear or consistent annual or seasonal difference or change for the species. Body size does not change or cycle during the year or over time in these populations. Females are always larger than males and *D. melanogaster* is always larger than *D. simulans* when a difference is found. Females may be larger than males due to their reproductive roles. Body size is an important fitness character and is important to correlate with the other traits in this study. (See literature review for a discussion of this.)

Organisms must adapt to cumulative environmental stress in order to survive. Since desiccation survival is of the utmost importance to *Drosophila*, they must have developed or adapted existing traits in order to cope with this stress. However, not all species will respond in the same exact way. For the Laguna Verde populations of *D. melanogaster* and *D. simulans*, there is no clear seasonal difference or changing between the two seasons; nor is there an annual (changing over time) difference for any of the three measured traits. Possibly, the Laguna Verde species stay close to their maximum adapted stress tolerance all year round, not resorting to alternating genomes between dry and rainy seasons and back again. They may lack the genetic variance for this seasonal cycling.

The Laguna Verde populations of *D. melanogaster* and *D. simulans* show some similar and some different responses to the stress of desiccation. Generalizing, *D. melanogaster* is superior to *D. simulans* and females of both species are superior to their accompanying males for survival in a desiccating environment; *D. melanogaster* is also more vagile both over a short or an extended period of time; and females are larger than males for both species, but *D. melanogaster* is a larger fly than its sibling species.

In this study, it is interesting to find that the species of fly (*D. melanogaster*) which has a greater rate of survival in the desiccator is also the larger and, in the case of males, the more vagile fly. *D. melanogaster* is larger than *D. simulans* and is superior at resisting desiccation (even in the short term). Females are larger than males, and females are better at surviving desiccation in both species. This was also found by Levins (1969). In terms of desiccation survival, the best fly to be may be a female *D. melanogaster*. *D. melanogaster* males are also more vagile than *D. simulans* males; and, again, better at surviving in a desiccating environment. The finding that larger flies are found to be more resistant to water loss than smaller flies is in accordance with the surface area to volume ratio. Only Eckstrand and Richardson (1980) found an opposite result which may apply only to *D. mimica* that was studied. Therefore, a fly with a greater dispersal actually may be a more fit fly in a desiccating environment in this study. Although females were not used in the vagility experiment (for logistical reasons), the predicted "best" fly may be a large, active female *D. melanogaster*. Possibly, a larger fly (female vs. male; *D. melanogaster* vs. *D. simulans*) relates to more body reserves and therefore the fly takes a longer time to desiccate. Possibly, more active (vagile) translates to a fly that can move away from a hostile microenvironment to a more favorable one. And, possibly, *D. melanogaster* originally had the genetic variation to better adapt to the harsh conditions of desiccation than *D. simulans*. Levins (1969) found that adaptation to different climates occurs rapidly in broad-niched species (especially in *D. melanogaster*). According to Davidson (1988), the ability to withstand extremes of climate by a population is due to its genetic heterogeneity which is a reflection of the prior selective history of the area. Differences, seasonal cycling, and changes over time may depend largely on the amount of expressed genotypic variation in a population.

Expressed genotypic variation allows a population exposed to stressful desiccation levels the potential to undergo rapid evolutionary change (for example, during seasonal changes). Although there were some small differences in expressed genotypic variance in desiccation resistance among some of the collections and between *D. melanogaster* and *D. simulans* of the Laguna Verde populations of these species, the level of expressed genotypic variance for the trait was exceptionally low. This contrasts with several studies of both natural and artificial populations (Parsons, 1970, 1973, 1981, 1987; McKenzie and Parsons, 1974; Ringo and Wood, 1984; and Hoffmann and Parsons, 1989a). (See literature review for a discussion of these works.)

In some of these studies, the high levels of genotypic variance for desiccation resistance may reflect the combining of gene pools from various strains or natural populations (Parsons, 1970; McKenzie and Parsons, 1974; Ringo and Wood, 1984; and Hoffmann and Parsons, 1989a). In the studies that examined single natural populations (Parson, 1970; McKenzie and Parsons, 1974), the contrast may reflect different ecological or evolutionary histories from the Laguna Verde populations.

There were no differences in expressed genotypic variance in vagility among the collections and between *D. melanogaster* and *D. simulans* of the Laguna Verde populations of these species. This also contrasts with several studies of both natural and artificial populations (Narise, 1962; Rockwell, 1979; Rosa *et al.*, 1984; Rockwell and Levine 1986a; and Rockwell and Levine, 1986b). (See literature review for a discussion of these works.) The high levels of genotypic variance for vagility in some of these studies may reflect the combining of gene pools from various strains or natural populations (Narise, 1962). For the single natural populations studies (Rockwell, 1979; Rosa *et al.*, 1984; Rockwell and Levine 1986a), the contrast may reflect different ecological or evolutionary histories from the Laguna Verde populations.

There were no differences in expressed genotypic variance in body size among the collections, between *D. melanogaster* and *D. simulans* of the Laguna Verde populations of theses species, and between the sexes contrasting with several studies of both natural and artificial populations (Reeve and Robertson, 1953; Prout, 1958; Barron, 1982; Curtsinger, 1986; Coyne and Beecham, 1987; Prout and Barker, 1989). (See literature review for a discussion of these works.) Again, in some of these studies, the high levels of genotypic variance for body size may reflect the combining of gene pools from various strains or natural populations (Prout, 1958; Curtsinger, 1986; Coyne and Beecham, 1987; Prout and Barker, 1989), and in the studies that examined single natural populations (Reeve and Robertson, 1953; Barron, 1982; Coyne and Beecham, 1987; Prout and Barker, 1989), the contrast may reflect different ecological or evolutionary histories from the Laguna Verde populations.

Relatively little genetic variation was detected for desiccation resistance, and no genetic variation was detected for vagility or body size in the Laguna Verde populations of *D. melanogaster* and *D. simulans*. This is in contrast to the other stated studies, but artificial selection, with its attendant inbreeding, can often uncover additive genetic variance not normally expressed (Falconer, 1960). This could mean that selection has eroded additive genetic variance for these traits. Alternatively, selection could have modified the genetic architecture such that additive genetic variance is not expressed as phenotypic differences among isofemale lines reared in the same environment (Rockwell, 1980). Finally, it is conceivable that these populations are inbred, perhaps as the result of passing through genetic bottlenecks. In such a case, all traits would display reduced variance, a situation not likely for these species.

Correlation analyses were performed for each species for each sex to determine the overall patterns of relationship among the traits. General patterns suggest that for *D. simulans* males and females, larger flies die more quickly than smaller ones at 12 and 24 hours in the desiccator. This is in contrast for *D. melanogaster* for which there is no obvious general pattern.

To further investigate the relationships, the analyses were performed for each year, species, sex combination. *D. melanogaster* males show no clear pattern of correlation among the traits for either of the two years suggesting that size and vagility do not affect their ability to survive in a desiccating situation. The results show that smaller *D. melanogaster* females in year=2 survive longer in a desiccating situation than their larger counterparts except under extreme conditions in which all the flies die (at 24 hours). After the initial stage of desiccation stress and before the extreme conditions are reached, *D. simulans* males may have a survival advantage by having a smaller size and being less vagile.

Analyses were performed for each month, species, sex combination. Overall, less vagile *D. melanogaster* and *D. simulans* males in month=1 (but not for month=7) survive longer in a desiccating situation. This may suggest seasonal variation. Vagility may make a difference for desiccation survival when the conditions are drier (month=1). No distinguishable pattern of vagility and size with desiccation resistance can be recognized for seasonal differences.

The general pattern that smaller flies survive longer in a desiccating environment may seem unusual at first. After all, insects have a large area to volume ratio because of their small size. Therefore, smaller flies should desiccate more quickly than their larger counterparts. Larger male *Drosophila* have been shown to have an advantage in mating (Ewing 1961, 1964; Partridge and Farquhar, 1981, 1983; Hoffmann and Cacoyianni, 1988; Farquhar, 1983). Female *Drosophila* have been shown to survive longer than male *Drosophila* in a desiccating environment. Investigators have assumed that this sexual dimorphism is due to the larger size of the females. A larger fly can carry more water and has a smaller surface to volume ratio lessening evaporation of the internal storage of water. But larger size may not be as important as other combined traits at lessening water loss in a desiccating environment. Water loss may be more influenced by closing of the spiracles (Fairbanks and Burch, 1970; Arlian and Eckstrand, 1975; Eckstrand, 1981) and behavior

(Eckstrand and Richardson, 1980), or by drinking large amounts of water (Arlian and Eckstrand, 1975). Although female flies survive longer in a desiccating situation than their male counterparts, it may not be due to their larger size; but due to other traits such as behavior.

Since the results suggest that less vagile flies survive longer, a more active fly would use its internal stores of energy, and would require their replacement. Possibly, male flies are more active (more vagile) than female flies in order to seek a mate. Females if less vagile, may be awaiting a male to find them. Females may be more vagile at the time of egg laying in order to find a suitable spot to lay their eggs, but may be less vagile at other times. Therefore, one might predict that the smaller males would display a greater vagility and lower desiccation survival than the larger females.

The result that desiccation resistance and vagility are negatively correlated when a significant difference exists may have an environmental explanation. A fly with a great vagility will most likely travel through different microhabitats, each exhibiting a different humidity. If this fly is also the fly with a greater desiccation resistance (compared to the less vagile flies), then the fly can afford explorations into more desiccating environments. A fly with a lower desiccation resistance would need to remain in a favorable environment. Chance explorations into other microhabitats would be a gamble. Therefore, a fly with a lower desiccation resistance would also be a fly with a lower vagility as found in this study.

CONCLUSIONS

Both means and genetic variances of the traits of desiccation resistance, vagility, and body size were used in the evaluation of the sibling species *Drosophila melanogaster* and *D. simulans* from Laguna Verde, Veracruz, Mexico. Annual, seasonal, species, and sex differences and changes were examined. Patterns of correlation among the traits for differences in these factors were also investigated. Whether any changes were concordant across the species were of interest.

Significant differences between the species exist. *D. melanogaster* is superior to *D. simulans* at resisting desiccation at all time intervals. *D. melanogaster* is also a larger fly, and its males are more vagile. These may reflect different evolutionary histories for the two closely related species. The species showed no annual or seasonal differences or cycling. Which is not surprising considering the exceptionally low, or lack of, expressed genetic variance for the traits for the species. Whether additive genetic variance has been lost, or reduced by the populations passing through a genetic bottleneck, or is not expressed, all the traits display a reduction in variance. The Laguna Verde populations of these sibling species may remain at their maximum adapted stress tolerance all year round. Lacking the genetic variance, they do not cycle between alternating genomes from dry season to rainy season and back again; nor should they change over time with respect to these traits.

Significant sex differences also exist, showing that female *Drosophila* are superior to their male counterparts for surviving in a desiccating environment, and display a larger body size. Female flies may need a greater desiccation resistance to ensure the survival and successful depositing of their eggs. A male *Drosophila* may live a more precarious existence, gambling with survival for the sake of mating and delivering its share of genetic material.

Surprisingly, at first, a more desiccation resistant fly is also the more vagile fly, and a smaller and more vagile fly is also a better fly at surviving in a desiccating environment.

However, several traits together, not just one, may confer an adaptive advantage for desiccation survival. A lower desiccation resistance may be environmentally linked to a lower vagility by restricting a fly to a favorable microhabitat. Vagility and body size may be just two of many traits that contribute to survival with respect to desiccation stress or regulating water loss. If a male *Drosophila* needs to be more vagile to ensure the continuation of its prodigy, it will need to use other traits in concert to combat desiccation. Its size alone is not the determining factor for its desiccation survival. Several traits together, whether physiological, behavioral, or morphological may allow *Drosophila*, and insects in general, to survive longer in a desiccating environment. A complex phenotype which has physiological, behavioral, and morphological components contribute to survival with respect to desiccation stress.

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