This is to certify that the

dissertation entitled

SPECIALIZATION IN POECILOCHIRUS CARABI,
A PHORETIC MITE

presented by

JONATHAN MILO BROWN

has been accepted towards fulfillment
of the requirements for

DOCTORAL degree in ZOOLOGY

Major professor

Date 7/26/89

SPECIALIZATION IN *POECILOCHIRUS CARABI*, A PHORETIC MITE

By

Jonathan Milo Brown

A DISSERTATION

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

DOCTOR OF PHILOSOPHY

W.K. Kellogg Biological Station
and
Department of Zoology

1989

ABSTRACT

SPECIALIZATION IN POECILOCHIRUS CARABI, A PHORETIC MITE

By

Jonathan Milo Brown

This study focuses on the role of ecological specialization in determining the local diversity of a phoretic mite species group, Poecilochirus carabi (= P. necrophori Vitz.)[Acari:Parasitidae]. The alternative "habitats" for these mites are a number of carrion beetle species of the genus Nicrophorus [Coleoptera:Silphidae], with which their reproduction and dispersal is closely linked. The close relationship between mites and beetles means that beetle community structure strongly influences the factors determining the evolution of ecological specialization in the mites. I describe the correlations between behavior, morphology and life history which indicate that specialization on a subset of beetle species occurs at two sites in lower Michigan. The adaptive nature of local specialization is tested in experiments comparing the fitness of specialist mites in association with their preferred and non-preferred beetle species. Studies of mite generation time, and its consequences for certain components of fitness, strongly suggest that different beetle species can represent different habitats from a mite's point of view.

The high degree of variation between sites in the degree and nature of specialization suggests that mites evolve to use beetle species in response to local conditions of selection. Knowledge of geographical variation in mite specialization, in concert with an understanding of the community ecology of their host species, allows

me to generate evolutionary hypotheses which explain the distributions of specialist mites. These hypotheses differ in the mode of speciation proposed for a newly-discovered mite specialist species, but both hypotheses stress the importance of local evolution of beetle species use in determining the distribution of specialist species of mites. One hypothesis proposes that specialization determines the competitive environment a mite experiences, which may affect the ability of a specialist to persist, even when its host species is present. A second hypothesis stresses the importance of population structure in determining whether intraspecific polymorphisms in beetle species use may evolve and lead to divergence, or speciation. This hypothesis is explored in a theoretical model, which examines the effect of migration on the spread of locally diverged specialist species to areas in which local conditions do not favor the evolution of reproductively-isolated habitat specialists.

ACKNOWLEDGEMENTS

I thank my committee members, past and present, Dave Wilson, Steve Tonsor, Earl Werner, Guy Bush, Susan Kalisz, Barry OConnor and Don Straney for their guidance during these many years. Dave Wilson provided the intellectual and financial support for this project to work. Thanks to Wes Knollenberg for providing expert carrion beetle advice and assistance. M.L. Moraza and D.E. Johnston kindly identified morphological differences between the mite types. Dan Howard was instrumental in my getting all this going during my first year on campus. I owe many thanks to Mark McPeek, Rob Creed, Mathew Leibold, Craig Osenberg, Denise Thiede, Steve LaChance, and Scott Gleeson and the KBS faculty for discussion and criticism of my work over the years. A conservative thank you to John Gorentz for computational consultation. Carolyn Hammerskjold provided superlative library support. Thanks to C. Papazian for philosophical guidance and a few good recipes. The work was financially supported by an NSF Graduate Fellowship, NSF grant BSR 8320457 to D.S. Wilson, a Grant-in-Aid of Research from Sigma Xi (the Scientific Research Society), and an MSU College of Natural Science Continuing Fellowship.

It seems strange to dedicate a work on carrion biology to those you love most. But I must dedicate this, as everything of which I am proud, to Rebecca. This work is also dedicated to my parents, Bernard and Carol Jean Brown, both the finest examples of scholars.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

Page

CHAPTER 1. INTRODUCTION

The evolution of ecological specialization has been recognized as a central problem in evolutionary biology since the publication of Darwin's On the Origin of Species (1859) (see Futuyma and Moreno, 1988 for review). The idea that ecological specialization is at the root of evolutionary divergence traces back to Darwin, who viewed nature as providing a multitude of adaptive pathways, essentially specialized niches in our modern terminology, and saw natural selection as leading to the diversification of species into varieties which occupied these " . . . widely diversified places in the polity of nature . . ." (1859, p. 112). Intraspecific varieties, in turn, ". . . are species in the process of formation, or are, as I have called them, incipient species." (1859, p. 110) This view is reflected in a body of theory which proposes that a single phenomenon, i.e. selection for different trait values in different parts of the environment, may explain both intraspecific (genetic) variation and interspecific diversity.

The development of ecological models of speciation in the past several decades has emphasized the potential interaction between the evolution of ecological specialization and the splitting of lineages (Bush, 1975; Pimm, 1979; Rice, 1984). Environmental heterogeneity is the presumed basis for producing selection for different trait values in different parts of the environment, or disruptive selection (Mather, 1955). Disruptive selection is the central element in many theoretical models which seek to explain the maintenance of high genetic diversity in single-species populations (see Hedrick et al.,

1976 and Hedrick, 1986 for reviews). These models generally propose that different homozygote genotypes are most fit in different parts of the environment, leading under certain conditions to a situation of balanced polymorphism (Levene, 1953). Extending this paradigm to explain interspecific diversity, Maynard Smith (1966) showed that following the development of intraspecific polymorphism, the production of low fitness "hybrids" (i.e. heterozygotes) between the two specialized genotypes would result in selection for assortative mating. Complete linkage disequilibrium between the genes responsible for habitat fitness and assortative mating results in sympatric speciation, or "the establishment of new populations in different ecological niches within the normal cruising range of the individuals of the parental population . . ." (Mayr, 1947).

The importance of ecological specialization in the process in the generation of biological diversity has remained controversial, however, for several reasons. Much of the argument has concerned the sympatric nature of speciation, specifically questioning whether intrinsic isolating mechanisms are the initial steps in population subdivision (Futuyma and Mayer, 1980). A number of objections to this speciation mechanism rest on theoretical grounds. First, the maintenance of genetic polymorphisms requires strong divergent selection between habitats (i.e. strong disruptive selection on the entire population). Second, recombination between fitness and assortative mating loci breaks down reproductive isolation between the subpopulations, and thus imposes a genetic constraint on the likelihood of sympatric speciation (Felsenstein, 1981). These two constraints become less important, however, when the models are

altered to reflect two aspects of many groups of organisms: (1) Organisms use habitats in a biased manner, i.e. they select habitats. (2) Populations are not pan-mictic, but rather matings occur in or near the habitat (Bush, 1975). This last condition allows assortative mating to evolve as a correlated character with habitat selection, breaking the antagonism between selection and recombination (Rice, 1985, 1987). Although the strength of divergent selection between habitats still affects the rate of evolution, strong selection is not necessary to maintain polymorphisms or to result in population subdivision based on differential habitat use (Diehl and Bush, 1989). Thus both the ability of organisms to accurately choose habitats and the linkage of habitat selection and mating are primary determinants of the outcome of evolution for specialization. Environmental heterogeneity may thus lead to a variety of outcomes, ranging from non-specialization, to the maintenance of ecological specialists within single populations (Wilson and Turelli, 1986), to the divergence of a single species into multiple ecological specialists, or **speciation by habitat specialization.**

Another set of objections to sympatric speciation by habitat specialization rest on the validity of empirical evidence for the process. Futuyma and Mayer (1980) summarize these objections, which center around the difficulty of inferring past events from current pattern of distribution. How can one know whether two specialists have speciated allopatrically and later become sympatrically distributed, then evolving to specialize on different habitats? Allopatric speciation has been proposed as the more parsimonious

hypothesis in these cases, although there are those that argue that evidence exists to separate the two hypotheses (e.g. Bush and Howard, 1986).

This study focuses on the role of ecological specialization in determining the species diversity of a complex phoretic mite community associated with carrion. The alternative "habitats" for these mites are a group of carrion beetle species with which their reproduction and dispersal is closely linked. The close relationship between mites and beetles means that beetle community structure strongly influences the factors determining the evolution of ecological specialization in the mites. Variation in beetle community structure over space makes this system ideal for comparing the local evolution of specialization in the mites under different conditions of selection and population structure. The geographical patterns of variation in ecological, behavioral and morphological characters allow me to generate evolutionary hypotheses which explain the current distributions of specialist mites. While the goal of this study was not to discriminate conclusively between sympatric and allopatric hypotheses of speciation in this group, the results provide predictions for future testing of these hypotheses. My goal was rather to demonstrate the importance of local evolution for ecological specialization in the species interactions which determine local community structure.

NATURAL HISTORY

Poecilochirus carabi (= P. necrophori Vitzthum) [Acari:Parasitidae] is a large (approximately 1mm body length) mite that feeds on carrion and other carrion-associates, such as nematodes and fly eggs (Springett, 1968; Wilson and Knollenberg, 1987). It disperses from one carcass to another by riding on silphid beetles, which also feed on carrion. Use of another species for transport is called phoresy. P. carabi is one of many species of such phoretic mites found on silphids (Wilson and Knollenberg, 1987). The latest taxonomic treatment of the Poecilochirus describes 7 species in the genus (Wise et al., 1988), although there has been much confusion in the literature, due to apparent synonymies. A discussion of the controversies and their bases is given in Hyatt (1980). While mites of this genus have been described in a wide variety of habitats (from carrion and dung to the nests of wasps), the species P. carabi has been described primarily in association with carrion beetles (Coleoptera: Silphidae). Most often they have been described in association with beetles of the silphid genus Nicrophorus, the so-called burying or sexton beetles (Hyatt, 1980; Korn, 1983; Karg, 1971; Micherdzinski, 1969; Springett, 1968; Turk and Turk, 1952; Willman, 1943; Cooreman, 1943; Neumann, 1943; Vitzthum, 1930; Kneissl, 1915). The suggestion of a strong mutualistic relationship between Poecilochirus mites and Nicrophorus beetles was made by Springett (1968). Wilson and Knollenberg (1987) demonstrated a neutral to weakly beneficial effect of the entire mite community on the reproductive success of beetles.

The relationship between P. carabi and Nicrophorus beetles is significant given the specialized life-style of this beetle genus. These beetles are excellent fliers and locate carcasses by olfaction. Unlike other genera in their family (e.g. Oiceoptoma) which reproduce on all sizes of carrion, Nicrophorus beetles reproduce on small carrion (10-70g) such as birds and mice. While they are abundant at large carcasses, they bury small carcasses and use them as a food source to raise their offspring (but see Peck, 1986). Typically, a single pair of beetles buries the carcass, first dragging the carcass under the leaf litter and then excavating the soil from underneath the carcass. The beetles form the carcass into a ball, which lies in an underground "burial chamber," 3 to 8 cm below the soil surface. After the eggs are laid in the surrounding soil, the beetle adults remain in the chamber and wait for their offspring to hatch. During this period they tend the carcass, spreading anal secretion over the surface of the ball, which may help in its preservation from invaded microorganisms, particularly fungi. Both sexes may then feed the offspring by regurgitation, a step apparently necessary for first instar survival, at least in some species (D.S. Wilson, pers. comm.). The female parent usually remains until the 4th instar larvae begin to disperse into the soil to pupate. The male parent generally leaves before this time. Both sexes are able to raise additional clutches on new carcasses in the lab (Wilson et al., 1984; Brown, pers. obs.), and presumably do so in the field if they can find and compete for a new small carcass. While this description represents a typical life cycle for Nicrophorus beetles, individual species may deviate significantly in the number of beetles which participate in carcass burial, the size of carcasses used and the details of

parental care (Peck, 1986; Trumbo, unpubl.; Bartlett, 1987; Brown, pers. obs.).

The reproductive cycle of P. carabi is closely tied to the patterns of parental care in these beetles. The mites ride on beetles in their only phoretic lifestage, the deutonymph (or preadult). When beetles arrive at large carcasses to feed, mites may abandon their carrier to feed on the carcass, returning to the same or a different carrier. Korn (1983) describes the feeding behavior of European P. carabi deutonymphs in detail. As the beetles begin to bury a carcass, the mites leave the beetles and swarm over the carcass, feeding where possible on fly eggs and carcass liquids. After 24-48 hours they molt to adults (males and females), mate and lay eggs in the soil surrounding the chamber. The larvae emerge and feed on the carcass, eventually molting to the protonymph and then to the deutonymph lifestage. As deutonymphs, they disperse on either the male or female beetle, or accompany the beetle larvae to their pupal chambers. Males are preferred to females by mites ready to disperse (Brown, unpubl.). This preference sometimes results in huge numbers (>500) of deutonymphs attempting to disperse on a single beetle parent (Springett, 1968; Brown, pers. obs.). This process is summarized in Figure 1.1.

Nicrophorus are also notable for the number of species which are found sympatrically. Nicrophorus species are typically season and habitat specialists, which permits coexistence of many species requiring an identical resource, i.e. small carcasses (Anderson, 1982; Wilson et al., 1984). In northern Michigan four species are

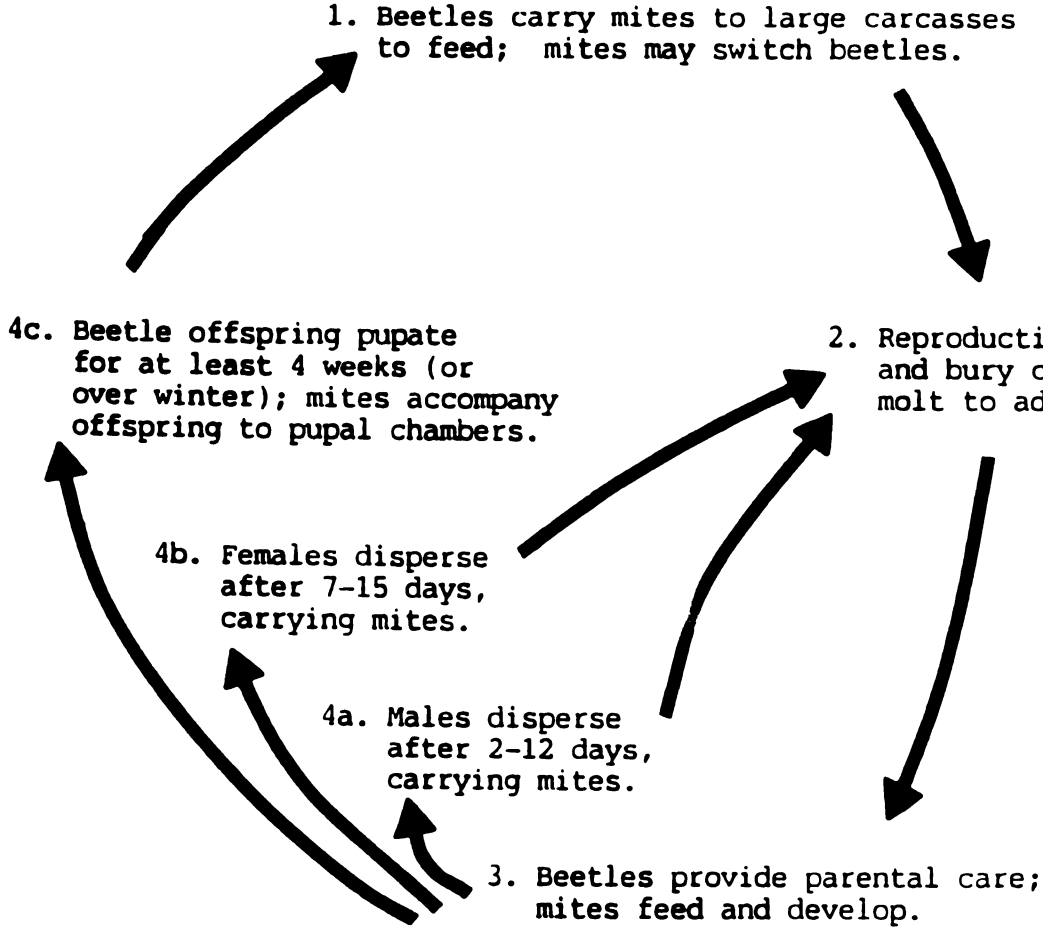


Figure 1.1 Reproductive cycles for Nicrophorus and P. carabi. Both reproductive and non-reproductive beetle species feed at large carcasses, and mites may transfer between beetles at these times. Mites must reach the phoretic (sclerotized) lifestage, the deutonymph, before dispersing. Mites may disperse from burial chambers on either the male or female parent or the offspring beetles, but dispersing on the latter two involves delaying the next opportunity for reproduction.

commonly captured in wooded areas, while 2 more species (N. pustulatus and N. vespilloides) are rare and poorly studied. Another species, N. marginatus, is captured solely in open habitats (e.g. old fields). Figure 1.2 illustrates the times of year during which the common woodland beetle species are flying but non-reproductive (i.e. found feeding at large carcasses only) or are reproductively active. Since all beetle species meet at large carcasses to feed, P. carabi mites are potentially phoretic on 7 species of Nicrophorus which differ in behavior, seasonality, size, etc. Thus large carcasses act as 'arenas' where selection between beetle species can occur.

While most Nicrophorus communities are diverse, species diversity and composition varies geographically. Both biotic and abiotic factors may influence species distributions, although these factors are in need of study (Peck and Kaulbars, 1987). Wilson et al. (1984) have demonstrated the role of temperature-dependent competition in defining the community structure of Nicrophorus communities at two study sites in lower Michigan, the University of Michigan Biological Station (UMBS) in Pellston (lat. 45^{o} 34') and Michigan State University's Kellogg Biological Station (KBS) in Hickory Corners (lat. 42^{o} 34'), 315 km south of UMBS. Three of the four species shown in Figure 1.2 are common in wooded areas at both sites; the exception is N. defodiens which is found at UMBS, but not at KBS. N. defodiens and N. orbicollis reproduce during the same period and thus compete for small carcasses at UMBS. These two species differ, however, in both body size and diel activity patterns. Typically, the smaller, crepuscular N. defodiens find carcasses before the nocturnal N. orbicollis. However, the latter species is able to

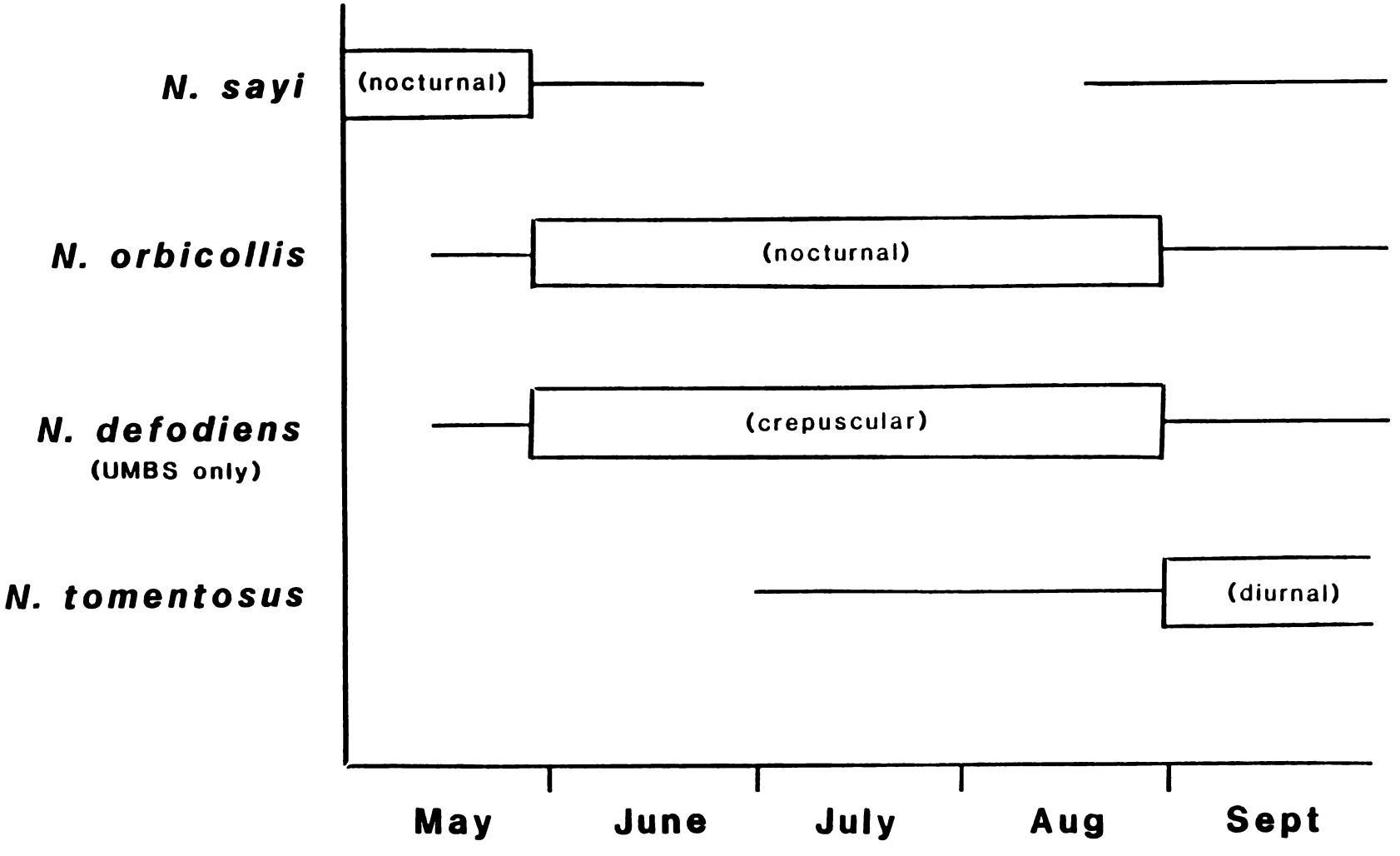


Figure 1.2 Phenologies of common woodland Nicrophorus in Michigan. Solid lines refer to times when beetles are found in pitfall traps but not at reproductive carcasses (i.e. they are in reproductive diapause). Boxes refer to reproductive periods. At other times beetles are either in the forest litter or dormant underground. Species actively search for carcasses with the diel pattern shown inside each box. (Adapted from Wilson et al., 1984)

locate the carcasses being buried by N. defodiens and, due to their larger size, appropriate them for their own reproduction. Neither species searches for carcasses when the temperature drops below 13° C., but since N. defodiens fly earlier in the evening, they are able to find and hold carcasses on nights when the temperature drops below about 13° C. after dusk. Thus, the two species coexist at UMBS (the northern site), but not at KBS (the southern site) where such nights are infrequent during the summer, and N. orbicollis is the solitary summer breeding species (Wilson et al. 1984). Due to these interactions, mites at the two sites must choose between different sets of beetle species.

In summary, three aspects of this phoretic system make it ideal for studying the evolutionary consequences of ecological specialization: (1) The mites have the opportunity to choose between several beetle species which may differ in their ecology and behavior. The beetle species may thus represent different habitats to the mites. Ecological and behavioral differences between beetle species can translate to divergent selection pressures on mites reproducing in association with different beetle species. (2) The reproductive cycles of the mites and beetles are closely linked, i.e. mites mate in their chosen habitats. (3) The species diversity and composition of the beetle community change geographically. I will argue that these changes have affected the evolution of specialization by changing the ability of mites to choose habitats, the selection differential between habitats and the degree of mating in the habitat of choice. Differences between local communities in specialization have consequences both for intraspecific population

differentiation and interspecific competitive interactions.

I have divided my description of the patterns and processes of specialization in P. carabi into 4 chapters. In Chapter 2 I describe the basic patterns of behavior, morphology, and mating success which indicate that at 2 sites in Michigan, mites specialize on particular beetle species. In Chapter 3 I present a set of experimental results which demonstrate that these specialists have higher fitness in their preferred than in their non-preferred habitats, i.e. that specialization is adaptive. I also consider two evolutionary hypotheses which explain geographical differences in the local evolution of specialization. These hypotheses differ in the mode of speciation proposed for a newly-discovered mite specialist species. In Chapter 4 I develop a computer simulation model of speciation by habitat specialization and consider the effect of migration on patterns of local specialization. This model demonstrates the roles of divergent selection in different habitats, habitat selection and mating in the habitat on the level of divergence between habitat specialists. It also explores the factors which may prevent the spread of specialist species to areas where speciation is not favored by local conditions. I discuss how the results of these simulations affect my interpretation of geographical patterns of specialization in P. carabi. Finally, in Chapter 5, I present preliminary results of morphological and behavioral analyses from sites other than KBS and UMBS and discuss their relevance to the understanding of the evolution of specialization in P. carabi.

CHAPTER 2. EVIDENCE FOR SPECIALIZATION ON DIFFERENT BEETLE SPECIES

The first step in studying the evolution of ecological specialization is to identify differences within or between populations in the degree or nature of specialization, i.e. the range of resources used by the species being studied. In 1982, Wilson reported evidence of a genetically-based preference for beetle species in Poecilochirus carabi. Mites which had chosen KBS N. tomentosus or N. orbicollis in choice tests were allowed to reproduce along with both of these beetle species, and mite progeny were then tested for preference. Mites showed the same preference as their parents, regardless of the associated beetle species (Table 2 in Wilson, 1982). P. carabi at KBS, then, appeared to be one of two specialist types. This chapter describes experiments and observations which provide further evidence of specialization by mites at both study sites, but also document differences between the nature of specialization at UMBS and KBS. First, at both sites the average mite densities were determined for each beetle species. If mites are specializing on particular beetle species, differences in the number of mites found on the various available beetle species should be apparent. I then performed choice tests to determine whether differences in associated mite densities between beetle species could be due to active preference by the mites. Since beetles of many species meet at large carcasses, and mites disembark to feed at these carcasses, one would expect that specialist mites should be able to discriminate between the available beetle species. The discovery of two distinct behavioral

types at KBS led Wilson (1982) to speculate that these types may be distinct specialist host races or species. In order to test this speculation, I performed morphological comparisons and mating trials between these two behavioral types. UMBS mites were also analyzed morphologically, and compared to KBS mites of both behavioral types. The results of these experiments provide information on the correlation between behavioral and morphological traits across the geographical sites.

MATERIALS AND METHODS

I. Densities of P. carabi on Nicrophorus species.

The beetles and mites were collected using pitfall traps baited with rotten fish (smelt). Traps consisted of a 1 X 1 m plywood platform with a 15 cm hole cut in the center covered by coarse screen. A steel (#10 size) can was suspended under the hole with an elastic band. Traps were hung in trees to discourage vertebrate scavengers. Beetles attracted to the bait land on the platform and fall into the cans in the same species proportions as cans buried at ground level (Wilson, unpub.). At KBS sampling sites were located in woodlots near the Biological Station or 26 km north in the Barry County State Game Area. These wooded areas contain mesic oak-hickory communities. Sampling sites at UMBS ran along two transects (Grapevine Point and Pine Point) of northern mixed hardwood-conifer forest adjacent to the Douglas Lake Station.

Mite densities were estimated by counting mites on beetles held under a magnifier. Densities are those after beetles and mites have

mixed in the collection boxes at least 24 hours, which allows any mites which have disembarked from their beetles in order to feed to reembark on the beetles. The species, sex and body of each beetle was also determined. Beetles were sexed by examining genitalia under a dissecting microscope. Pronotum width, which is positively correlated with body weight (D.S. Wilson, unpubl.), was measured with dial calipers. I performed within beetle species of analysis of the relationships between beetle size, sex and P. carabi density using an analysis of covariance.

II. Preference of P. carabi for certain Nicrophorus species.

(a) Pairwise interspecific trials -- After collection from pitfall traps and mixing in collection boxes (approx. 30 beetles/4500 cm^3 shoe box) for 24 hrs, beetles and their associated mites were isolated from each other by placing them in small plastic tubs with moist paper towelling. Mites were then removed using a stream of CO_2 and a fine camel hair brush and placed in an empty tub with towelling. Mites from a single beetle species were tested for preference in pairwise tests by adding two miteless beetles, one of each of two species, to each tub and counting the densities on each beetle after a 24 hour period. Mite densities tend stabilize after 4-6 hours (Brown, unpub.). Although tests usually included the beetle species from which the mites were removed, mites were never tested with the individual beetles from which they were removed. Preference can be expressed for each pair of beetle species by the proportion of all mites on each species. For statistical purposes, preference was also expressed as the number of trials "won" by the beetle species from

which the mites were derived. (A "win" occurs when one beetle has more mites on it than the other beetle.) The number of wins was tested against the null hypothesis of an equal number of wins by both species by a chi-square test. The results reported here include trials run in 1981 by D.S. Wilson and J. Fudge and in 1985 and 1987 by the author. [Some of the 1981 trials did not allow calculation of the number of wins, so that statistical analysis could not be performed on the results.]

(b) Double-rounded choice trials -- I performed a number of pairwise interspecific choice trials on UMBS and KBS mites which extended past a single round of testing. After the first round (as described above), a new set of pairwise trials were performed, keeping track of the origin and first round behavior of a group of mites. In this manner the repeatability of beetle species preference could be determined. I performed such trials with mites from KBS N. orbicollis and N. tomentosus and UMBS N. defodiens and N. tomentosus.

III. Morphological analysis

The analysis of morphological variation in P. carabi was performed on two characters:

(a) **Body size** -- Body size was determined by measuring the length and width of the area covered by the dorsal (podonotal and opistonotal) shields, using an ocular micrometer on unmounted specimens under a stereo microscope. KBS mites which prefer N. orbicollis and N. tomentosus ('orbicollis-' and 'tomentosus-specialists', respectively) were transferred to pairs of

beetles of both species. The mites and beetles were then allowed to reproduce on 20-25 g dead mice in styrofoam buckets kept in a temperature and light-controlled room (19^{o} C., L:D 16:8). The design was thus a 2x2 factorial crossing mite behavioral phenotype (TYPE) and associated beetle species (HOST). Ten mites were measured from each of 4 buckets in each treatment. In the statistical analysis, 10 mites from each group were considered as multiple samples from a single experimental unit (in order to remove any effect of relatedness on size). Length and width were used as response variables in a multivariate analysis of variance (MANOVA) to determine the effect of TYPE and HOST on size. 120 UMBS mites raised under identical conditions were also measured. Mites from UMBS N. defodiens were raised on N. defodiens and N. orbicollis, and mites from UMBS N. tomentosus were raised on N. tomentosus. The body lengths and widths of UMBS mites were compared to KBS mites using Tukey's multiple range test.

(b) **Dorsal setal pattern** -- Setal pattern was analyzed on the same group of mites as those used in the body size experiments. The lengths of 19 podonotal setae (see Table 2.3) were measured by ocular micrometer on mounted and cleared specimens under a compound microscope at 200X. Podonotal setae were chosen after observations indicated that they appear to vary between KBS mite types. From all sets of 19 lengths from each mite, a principal components analysis was performed (PROC PRINCOMP, SAS Institute Inc., 1985). Differences in setal size pattern (principal component scores) between KBS mite behavioral types raised on both species were analyzed by analysis of variance (ANOVA). Again, mites taken from the same bucket were

treated as samples from a single unit in the analysis. Mites from UMBS were compared to KBS types using Tukey's multiple range test.

IV. Mating trials

KBS mites which prefer either N. tomentosus and N. orbicollis were placed (in separate boxes) with reproductively active beetles, soil and a dead mouse. Over the next 24 hours, I removed from the boxes and isolated in petri dishes mites which were developing to adults but had yet to shed their exoskeleton. Since females produce no offspring unless mated after shedding the deutonymphal exoskeleton (Brown, unpubl.), these methods assured that virgin females were used. Molted males and females were then paired and transferred to individual plastic tubs with moistened topsoil and provided with fish ad libitum. Develop of offspring was followed for at least 10 days. In total, 7 T X T, 5 O X O, 5 T-male X O-female and 11 O-male X T-female crosses were performed in 3 sets over August-October 1987. [T refers to tomentosus-specialists, and O refers to orbicollis-specialists].

RESULTS

I. Densities of P. carabi on Nicrophorus species.

Mean densities of the mites on the various beetle species differed dramatically between sites and for N. sayi between sampling dates (Table 2.1). At UMBS N. tomentosus and N. defodiens have high average densities of mites (approx. 9/beetle), while N. orbicollis carry consistently low densities (0-1/beetle). N. sayi carry extremely high densities in September, but low numbers in early June. At KBS, both N. orbicollis and N. tomentosus carry moderate average densities

Table 2.1 P. carabi densities. Number of beetles (n), mean number of mites/beetle (Density), and standard error are given for four beetle species at both sites on the dates shown.

	N. orbicollis		N. tomentosus		N. defodiens		N. sayi	
Site	n	Density	n	Density	n	Density	n	Density
UMBS								
5/8/88		--		--		--	100	3.9 ± .3
6/1/87	32	.3 ± .1		--	33	9.9 ± .9	17	1.5 ± .5
8/10/87	40	.3 ± .1	41	9.9 ± 1.0	41	10.3 ± .9		--
9/8/87	16	.5 ± .2	31	9.6 ± .8	21	8.7 ± 1.5	10	22.9 ± 5.0
KBS								
7/30/85	80	4.0 ± .4	103	6.1 ± .6		--		--
5/1-4/88		--		--		--	84	2.9 ± .3

(approx. 5/beetle). The differences between mean densities (pooled over dates) were statistically significant (T-test, $p < 0.01$) for all species found at both sites. The difference in means between sites was not significant for N. sayi when the September 1988 sample was removed from the analysis.

Variation within a beetle species in mite density can be great (0-50+/beetle). Relative mite density is a weakly predictable trait of an individual beetle, with such factors as sex and size of the beetle correlated to mite density. Figure 2.1 shows the significant correlation of P. carabi density with pronotum width for N. orbicollis (Figure 2.1a) and N. tomentosus (Figure 2.1b). Table 2.2 gives the results of separate T-tests for intersexual differences in P. carabi density for 3 beetle species (N. tomentosus, N. defodiens and N. orbicollis) at the 2 sites. Males almost always carry more mites than females, but this difference is rarely significant at the sample sizes tested. Table 2.3 summarizes the analysis of covariance of mite density for KBS N. orbicollis and N. tomentosus. Both sex and size significantly affect mite density in N. orbicollis (Table 2.3a). The significance of both parameters is caused by a steep correlation of size with density in females (larger females carrying more mites, while there is no significant correlation of size with density in males (Figure 2.1b). Neither size nor sex has a significant effect on P. carabi density on N. tomentosus (Table 2.3b, Figure 2.1b).

II. Preference of P. carabi for certain Nicrophorus species.

(a) Interspecific pairwise trials -- Mites were found to exhibit distinct preference for certain beetle species. The attractiveness of

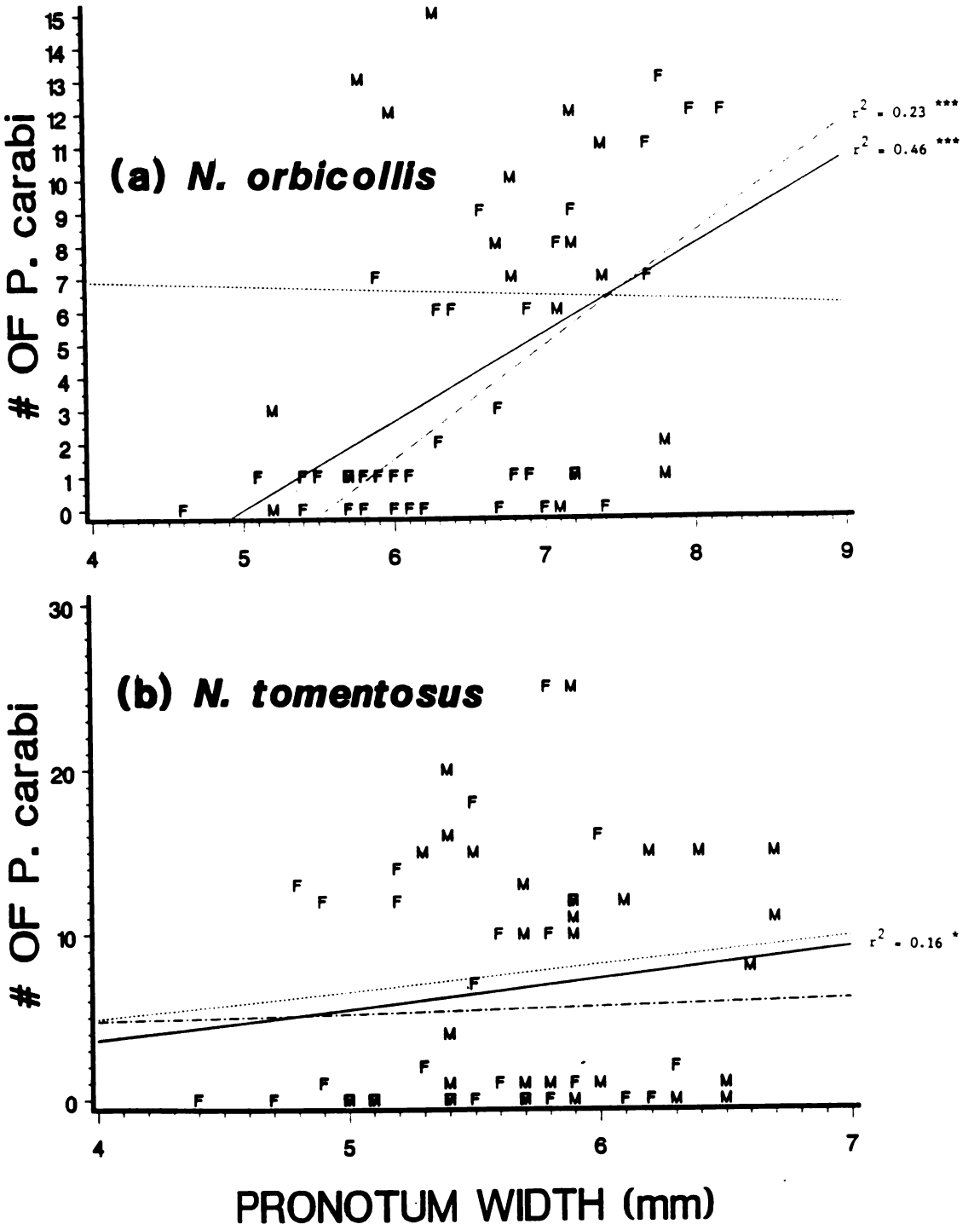


Figure 2.1 Plot of density of P. carabi versus beetle size on KBS (a) N. orbicollis and (b) N. tomentosus. Beetle size is represented by the width of the pronotum (in mm). M and F refer to male and female beetles, respectively. Least-square regression lines are shown for males (-----), females (—— - —— - ——) and both sexes combined (————). Statistically significant values of r^2 are shown (* denotes $p < 0.05$; *** denotes $p < 0.001$).

Table 2.2 Results of T-tests comparing the densities on P. carabi on 5 beetle species at KBS and UMBS. KBS beetles were caught on July 30, 1985. UMBS beetles were caught on June 1, 1986. (* $p < 0.05$, ** $p < 0.01$).

	Beetle sp.	Sex	n	Mean Density	s.e.	
KBS						
	N. orbicollis	F	43	2.8	0.6	**
		M	18	6.5	1.2	
	N. tomentosus	F	29	5.4	1.3	ns
		M	32	8.0	1.3	
UMBS						
	N. orbicollis	F	18	0.2	0.1	ns
		M	14	0.6	0.2	
	N. sayi	F	10	1.6	0.6	ns
		M	7	1.4	0.8	
	N. defodiens	F	18	8.1	1.1	*
		M	15	12.1	1.3	

Table 2.3. Analysis of covariance of the density of P. carabi with beetle sex and size. [* -- $p < 0.05$; *** -- $p < 0.001$]

(a) N. orbicollis

Source	d.f.	SS	F
Sex	1	104.5	6.8 *
Size	1	217.2	14.1 ***
Error	58	891.1	

(b) N. tomentosus

Source	d.f.	SS	F
Sex	1	61.5	1.19 ns
Size	1	14.0	0.27 ns
Error	58	3002	

beetle species varied both between sites and seasonally. The following hierarchy of preferences was determined for P. carabi at UMBS during the summer months (June-August, Figure 2.2a):

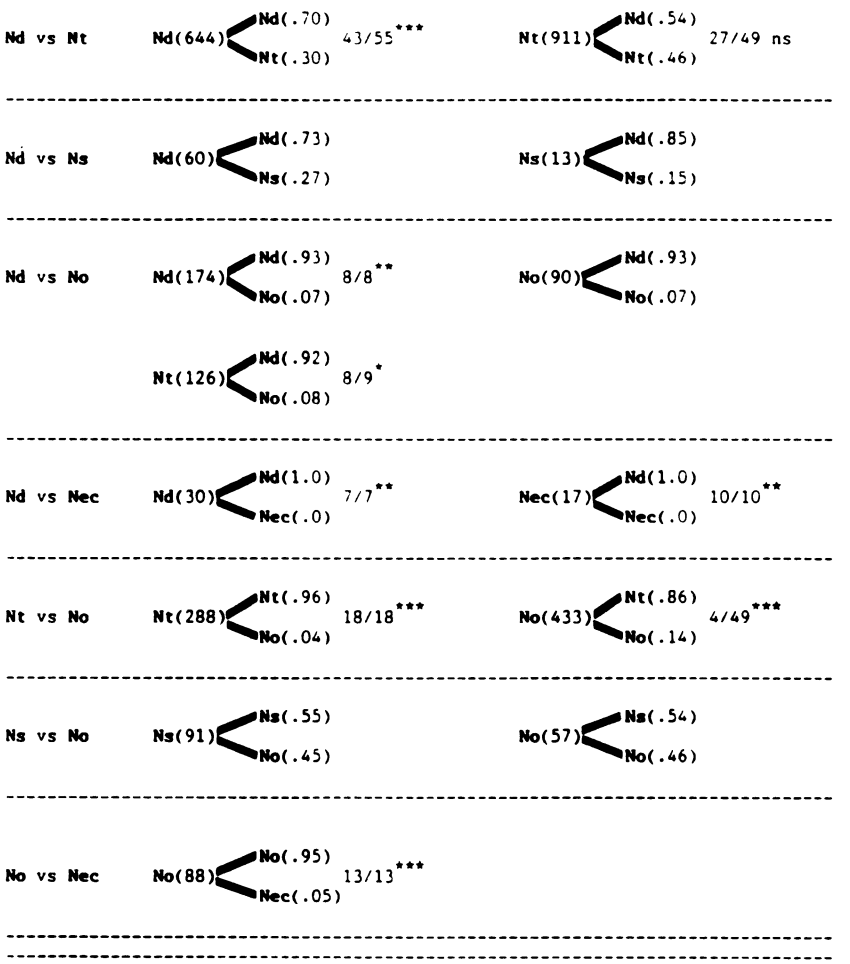
$$\text{N. defodiens} > \text{N. tomentosus} > \begin{matrix}\text{N. orbicollis} \\ \text{N. sayi}\end{matrix} > \text{Necrophila americana}$$

Necrophila americana is a silphid which does not bury carcasses for reproduction and rarely carries P. carabi. These preferences correspond to the relative densities of mites on the beetle species (Table 2.1), suggesting that mite behavior affects the densities of mites found on beetles in nature. This is particularly true in the case of N. orbicollis which is virtually shunned by mites at UMBS.

The above hierarchy, however, shifts seasonally. The results of preference experiments in September (Figure 2.2b) indicate that N. tomentosus is preferred over N. defodiens. Preference is correlated with the reproductive activity of the beetles: N. defodiens is preferred in August when it is reproducing, while N. tomentosus is preferred in September when it is reproducing and N. defodiens is in reproductive diapause. N. sayi also changes in attractiveness seasonally. It is rejected in early summer in favor of N. defodiens (Figure 2.2a), but when trials are run in September mites from N. sayi are as likely to choose it as the currently reproducing species, N. tomentosus (Figure 2.2b). This change in attractiveness corresponds to the dramatic difference in densities on N. sayi between June and September.

At KBS the relative status of N. orbicollis is rather different. Preference experiments involving N. orbicollis and N. tomentosus demonstrate that 80-99% of mites taken from one species return to that

(a) June - August



(b) September

Figure 2.2 Beetle species preference tests of mites and beetles from UMBS during (a) June through August and (b) September. Each pair of branches refers to a set of pairwise tests with mites taken from the beetle species at the node. The total number of mites used in each set is given in the parentheses at the node. The number in parentheses at the end of each branch is the proportion of all mites found on that beetle species in that set of trials. The fraction given at the right of each set of branches is the proportion of all trials won by the species from which all the mites in that set were derived (* p<.05, ** p<.01, *** p<.001, for chi-square test for expectation of equal number of trials won by each species). Because of different methods, some sets of trials could not be analyzed statistically. Nd, No, Ns, Nt, and Nec refer to N. defodiens, N. orbicollis, N. sayi, N. tomentosus, and Necrophila americana, respectively.

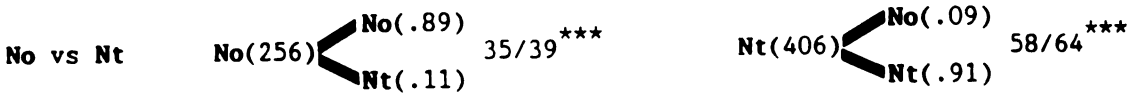


Figure 2.3 Beetle species preference tests using KBS mites and beetles, combined over several testing dates. See Figure 2.3 for an explanation of the symbols.

species when given a choice (Wilson, 1982; Fig. 2.3). In other words, KBS mites are of two behavioral types which consistently prefer either N. tomentosus or N. orbicollis. I shall refer to these types as tomentosus- and orbicollis-specialists, respectively.

(b) Double-round choice trials -- Double-round tests demonstrate the consistency of beetle species preferences by mites. Figure 2.4 shows such a test using mites from KBS N. orbicollis and N. tomentosus. These results demonstrate the strength and consistency of beetle species preference. While preference is strong, it does not result in the complete isolation of the behavioral specialist mites. This is shown in by the fact that some mites, which are originally found on one species, consistently prefer the other species over two rounds of testing. The lack of consistent beetle species preference among UMBS mites is shown in two double-round choice trials using UMBS N. defodiens and N. orbicollis (Figure 2.5). While overall preference between these two species shifts seasonally with the beetles' reproductive phases (Figure 2.2), the lack of strong discrimination between these two most attractive beetle species at UMBS is a contrast to the strong discrimination seen between N. orbicollis and N. tomentosus by the specialist mites at KBS.

III. Morphological analysis

Morphological analysis determined that the behavioral differences (i.e. beetle species preference) was reflected in two measures of morphology:

(a) Size -- Means of dorsal shield size for mites raised on different hosts and test of differences between groups are shown in

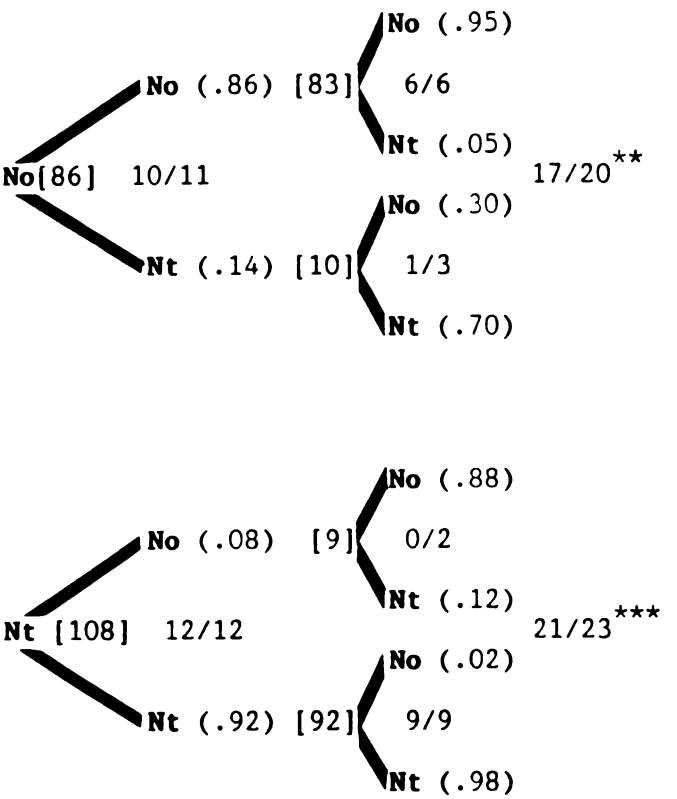


Figure 2.4 Double-rounded preference tests using beetles and mites from KBS, performed on August 8, 1981. See Figure 2.2 for an explanation of the symbols.

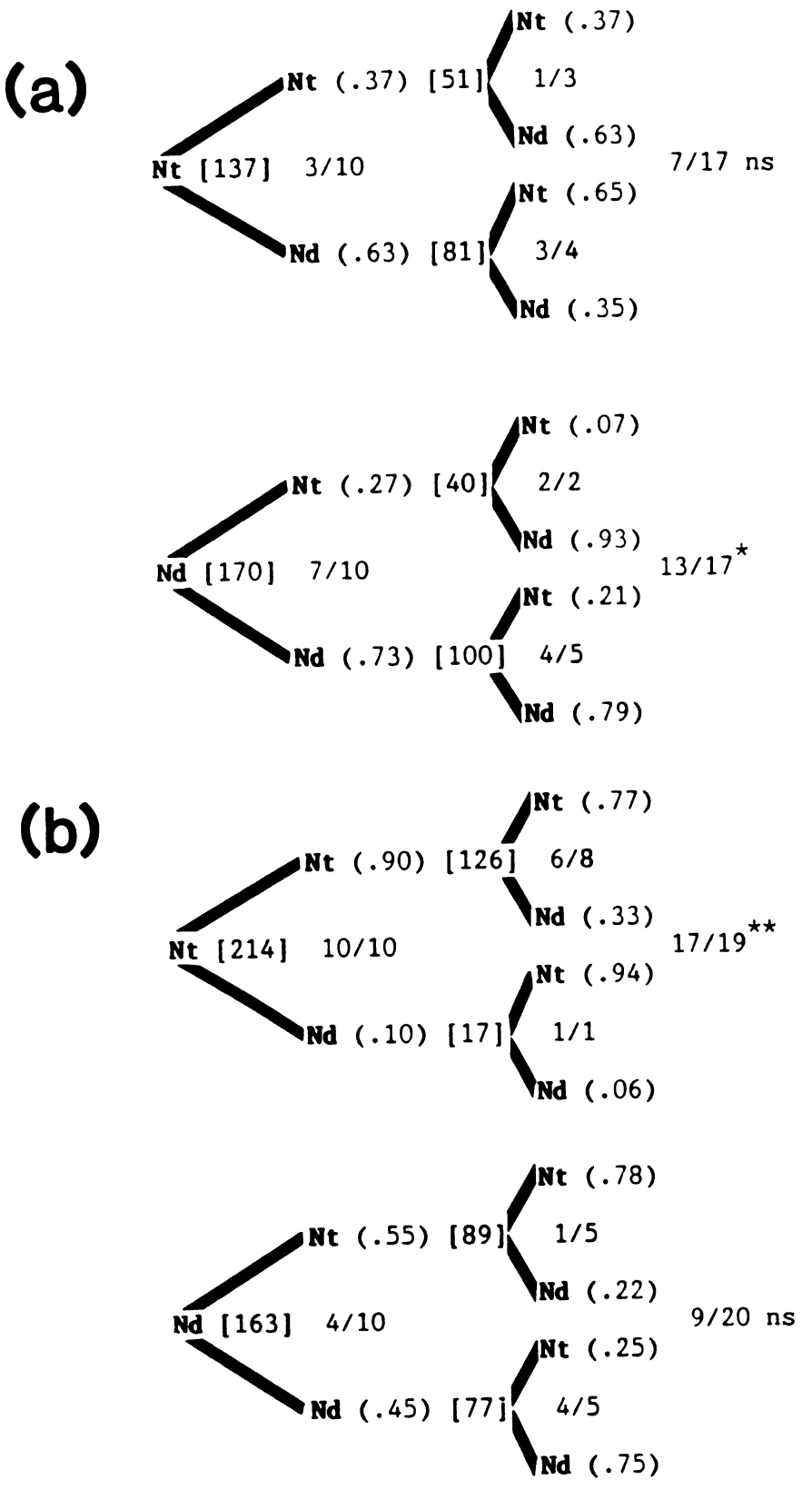


Figure 2.5 Double rounded preference tests using beetle and mites from UMBS, performed on (a) August 11 and (b) September 11, 1987.

Table 2.4. Mean sizes of the dorsal shield area (in mm) for P. carabi from KBS and UMBS, raised on different beetle species from the same site. Letters refer to groups not significantly different by Tukey's multiple range test ($p > 0.05$).

Mites from	Raised on	n	Mean Length	Mean Width
UMBS N. defodiens	N. orbicollis	40	0.992 de	0.744 b
UMBS N. defodiens	N. defodiens	40	1.000 cd	0.724 bcd
UMBS N. tomentosus	N. tomentosus	40	0.966 e	0.692 d
KBS N. tomentosus	N. tomentosus	40	0.990 de	0.700 cd
KBS N. tomentosus	N. orbicollis	40	1.024 bc	0.746 ab
KBS N. orbicollis	N. tomentosus	40	1.048 b	0.740 bc
KBS N. orbicollis	N. orbicollis	40	1.090 a	0.788 a

Table 2.4. When KBS orbicollis-specialist, tomentosus-specialist, and UMBS mites are compared (i.e. mites of the same type raised on different hosts are pooled), orbicollis-specialists are both longer and wider than the other two groups (Tukey's multiple range test, $p<.05$, Table 2.4). UMBS mite and KBS tomentosus-specialists are not significantly different in size. The MANOVA on KBS mites alone also indicates that orbicollis-specialists are larger than tomentosus-specialists (TYPE effect $p<.001$). However, there is a significant effect of HOST as well (HOST effect, $p < 0.001$). Mites raised with N. orbicollis are bigger than mites raised with N. tomentosus. This HOST effect applies to both mite types (HOST X TYPE, n.s.) and is not larger than the size difference between mite types. No significant differences were found between UMBS mites from different beetle species.

Setal morphology -- A summary of between type differences in setal length are given in Table 2.5. The eigenvectors generated by the principal components analysis (PCA) for setal lengths of 46 mites from KBS are shown in Table 2.6. A plot of PC2 vs. PC1 is shown in Figure 2.6a. The orbicollis- and tomentosus-specialists fall out into two distinct clouds of points. Analysis of variance on PC1 and PC2 indicates significant effects of TYPE only. Neither the effect of beetle species (HOST) nor the interaction (HOST X TYPE) are significant (Table 2.7). Figure 2.6b shows the plot when 30 mites from UMBS are added to the analysis. They clearly fall in the area of the tomentosus specialist cloud, even the rare mites found on UMBS N. orbicollis (plotted as asterisks). These latter mites do not differ significantly in either principal component from KBS

Table 2.5 Mean lengths of 19 podonotal setae for the two KBS behavioral specialists (O and T, 12 individuals/type). Also shown are the results of the analysis of variance for type and host (the beetle species with which each type was raised). Significant p values were adjusted by the Bonferroni criterion for multiple tests. Units are ocular micrometer units (= 0.00533 mm).

	Mean Lengths		Effects		
Seta	O-type	T-type	Type	Host	Morph X Host
i1	28.6	27.4	ns	**	ns
i2	16.6	22.6	***	ns	ns
i3	13.8	23.5	***	ns	ns
i4	36.9	37.3	ns	**	*
i5	11.5	18.6	***	ns	ns
z2	18.9	25.7	***	**	ns
z3	20.0	25.7	***	**	ns
z4	17.4	24.6	***	**	ns
z5	49.8	49.7	ns	***	**
s3	21.3	25.8	**	**	ns
s4	19.8	26.0	***	**	ns
s5	16.6	24.0	***	*	ns
s6	22.8	21.4	ns	ns	ns
r2	16.5	15.5	ns	*	ns
r3	61.1	62.4	ns	***	ns
r4	8.3	9.4	ns	ns	ns
r5	19.6	18.3	ns	ns	ns

Table 2.6. Eigenvectors associated with each setal length in the PCA (KBS mites only) for the first two principal components. These accounted for 62% and 17% of the variance, respectively.

SETA	PC1	PC2
i1	.047	.362
i2	.300	-.025
i3	.380	-.265
i4	.112	.198
i5	.302	-.257
i6	.190	-.112
z2	.310	-.017
z3	.232	.039
z4	.296	-.126
z5	.159	.427
z6	.185	-.090
s3	.224	.203
s4	.291	-.031
s5	.381	-.049
s6	.055	.206
r2	.055	.097
r3	.193	.578
r4	.058	.058
r5	.007	.193

Table 2.7. Analysis of variance on principal components scores based on setal lengths (see Table 2.6). Treatments corresponded to 2 KBS behavioral specialists (MORPH effect) each raised in association with N. tomentosus and N. orbicollis. 4 mites were measured for each replicate (3 reps/treatment) and treated as subsamples in the analysis. [*** -- $p < 0.001$]

Source	d.f.	SS	F	
HOST	1	312.3	2.8	ns
MORPH	1	3719.3	32.8	***
MORPH X HOST	1	48.8	0.4	ns
Error	7	793.4		

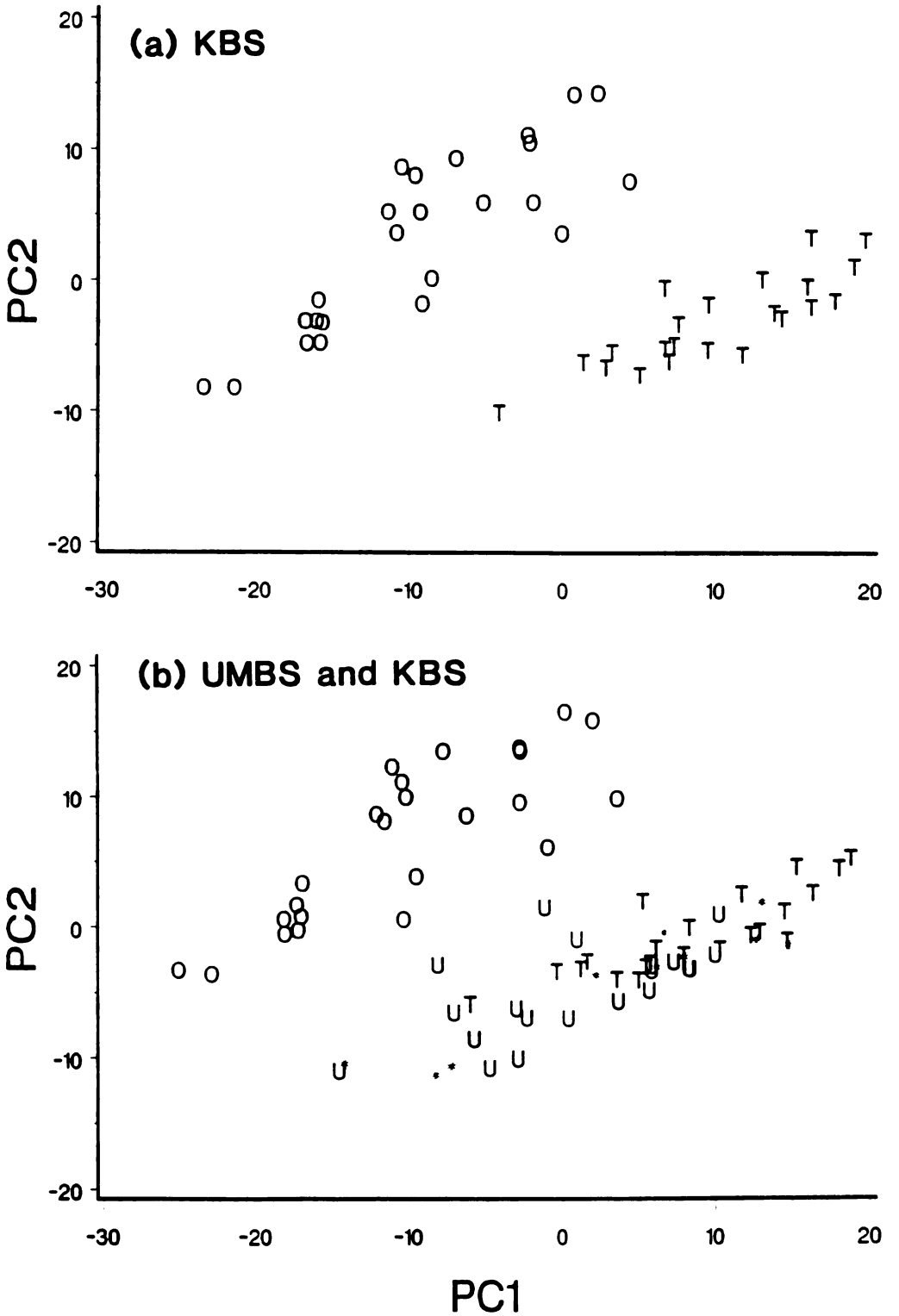


Figure 2.6 Principle component analysis on 19 setal lengths of P. carabi. Plots of PC2 vs. PC1 for (a) KBS mites only and (b) KBS and UMBS mites together. 'O' and 'T' refer to orbicollis- and tomentosus-specialist mites at KBS while 'U' refers to UMBS mites and '*' to mites found on UMBS N. orbicollis.

tomentosus-specialists or UMBS mites, while KBS orbicollis-specialists differ from all other mites in both these scores ($p<.05$, Tukey's multiple range test).

These analyses together indicate that the KBS behavioral specialists are morphologically distinct by two measures, size and setal morphometry. UMBS mites are similar to KBS tomentosus-specialists in both of these measures. The orbicollis-specialist morphology is not found at UMBS, despite the presence of N. orbicollis.

IV. Mating trials

Mating trials, run with KBS behavioral specialists, resulted in the following number of successful pairings (those producing offspring): T X T, 7 of 7; T-male X O-female, 0 of 5; O-male X T-female, 1 of 11; O X O, 4 of 5. Thus, 11 of 12 within type and 1 of 16 between type crosses were successful (significantly different by Fisher's exact test, $p<.001$). Since types were identified by behavior only, and mites do not exhibit absolute preference, it is possible that the one between type cross which was successful was actually between two identical specialist types.

DISCUSSION

These data indicate that the degree and nature of specialization is a geographically variable trait of P. carabi. At both of my study sites this mite is associated with at least 4 species of Nicrophorus and reproduces successfully in association with all of them (see

Chapter 3). P. carabi might then be considered a generalist in its phoretic relationships, using all carrion beetles as transport from patch to patch. Since all the Nicrophorus species use the same resource (small carrion) for reproduction, there is no need for a strict one-to-one species relationship. However, upon closer examination, preferences for certain beetle species can be detected. These preferences are reflected in the densities of mites found on different beetle species, and indicate specialization on the species with higher densities of mites. However, the nature of such specialization is different at the two study sites. At KBS, P. carabi consists of two distinct 'types' found on N. orbicollis and N. tomentosus in greatest densities. Each of the mite types is behaviorally and morphologically distinct. Due to "leakiness" in the preference system the two mite types are not completely spatially isolated, yet appear to be reproductively isolated, due to pre-zygotic (e.g. mating behavior) or post-zygotic (e.g. inviability of hybrid zygotes) mechanisms. These mite 'types' appear to be distinct species, each using a distinct habitat, i.e. different beetle species. At UMBS the patterns of beetle species use are very different. P. carabi at UMBS shifts its beetle species preference seasonally. While UMBS mites always prefer N. orbicollis, N. sayi and non-Nicrophorus silphids in favor of N. defodiens or N. tomentosus, they apparently shift their preference between the latter two species according to which is reproductively active. The double-round preference trials provided no evidence of strong species preference between these two species at UMBS, permitting a great deal of potential exchange of mites between the most highly preferred beetle species at UMBS.

The data reported here attest to the sophistication with which these mites can determine their habitats. Due to the large number of host species (both Nicrophorus and other Coleoptera) upon which this species has been reported, it might be assumed that they are not particularly discriminating in host use. In fact, P. carabi are not only able to distinguish different Nicrophorus species through the use of chemosensory setae (Korn, 1983), but perhaps can also detect the sex and reproductive status of individual beetles through behavioral or chemical cues. The significant correlation between beetle size and mite density in N. orbicollis (Figure 2.1b) could reflect an adaptive strategy of P. carabi, since larger beetle individuals always win in competition for reproductive resources, i.e. small carcasses (Wilson, et al., 1984). One aspect of host preference behavior which has yet to be explored in this system is whether mites adjust their choice decision based on the density of mites already occupying the host individual. This appears to be an ideal system to test these behavioral models.

The opportunity of mites to easily switch beetle species at large carcasses, coupled with the mobility of this mite species, opens the door to evolutionary flexibility in host use. Geographical shifts in beetle species preference shown here (see also Chapter 5) suggest that P. carabi has the potential to adapt to changes in its host's community through behavioral shifts. In the next chapter I describe tests of the adaptiveness of local beetle species preferences and relate the geographical changes in host use to changes in ecological interactions between the host species.

CHAPTER 3. THE ADAPTIVE NATURE OF SPECIALIZATION

The experiments and observations in Chapter 2 described differences between mites within and between my study sites in both behavioral and morphological characters. Differences between mites using different hosts at one site, KBS, were shown to be strong enough to suspect that these mites are reproductively isolated species. The goal of the experiments described in this chapter is to demonstrate that the behavioral specialization seen at the two sites is adaptive. If this is true, each of the behavioral specialists at KBS, the so-called "orbicollis- and tomentosus-specialists," should experience higher fitness in association with its preferred versus its non-preferred beetle species. UMBS mites should experience higher fitness in association with the mostly highly preferred beetle species at that site, N. defodiens and N. tomentosus. I have raised mites on their preferred and non-preferred beetle species and measured two components of fitness in order to test these predictions.

Fitness for a mite can be broken down into several different components which correspond to different events in the life cycle (Figure 1.1): (1) The probability that the beetle a mite rides on will find a small carcass. (2) Number and survivorship of the offspring mites in the burial chamber. (3) Survivorship of offspring dispersing on beetles from the burial site. My experiments will directly address only the second component of fitness, the number of offspring emerging from the burial chamber, which I shall refer to as reproductive success. These experiments also provide information on

the offspring mites' emergence pattern from the burial chamber, i.e. the distribution of offspring over male, female and offspring beetles. I will argue that the conditions under which mites emerge from the burial chamber, specifically the number of mites on each dispersal vector, can significantly affect the survivorship of offspring during dispersal. I will also describe experiments whose goal was to identify differences between mites in life history strategies. I will show how generation time is one factor which affects mites' emergence pattern from the burial chamber, and thus the dispersal component of fitness. Finally, using knowledge from these experiments, the morphological and behavioral data presented in Chapter 2, and information about the ecological interactions of the beetle species, I will outline evolutionary hypotheses which are consistent with the current distribution of mite specialists at my two study sites in Michigan and which, in particular, address the curious lack of an orbicollis-specialist mite at UMBS. Each hypothesis proposes that the evolution of local beetle species use, as a response to the local beetle community, is a primary determinant of species diversity of the local mite community by affecting the distribution of specialist mites over geographical space.

MATERIALS AND METHODS

I. Reproductive success experiments

Mites from both sites were tested for reproductive success with the common woodland beetle species of the local area. Since beetle species reproduce at different times of year, a standard protocol was developed and tests run on several different dates: Beetles were

trapped in pitfalls and allowed to mix in collection boxes for 24 hours. Males and females were paired randomly and isolated in tubs. P. carabi were removed from the pairs using fine forceps and replaced with 9-20 mites (average of 15) from either the same or a different beetle species. Beetle pairs were then placed in 8 liter styrofoam buckets filled with 7 liters of forest soil. A 20-25g frozen and thawed dead mouse (Mus musculus) was placed on the soil surface in each bucket. Buckets were kept in a temperature controlled room at 19^{o} C and L:D (16:8). Emergence traps (a cone of screen and a glass jar) were inverted over holes cut in the lids of the buckets. Beetles and their associated mites were caught as they abandoned their burial chambers and preserved in 70% ethanol. The number of mites emerging from the burial chamber on each parent and its offspring was determined later by counting under a stereo microscope. Thus, the total number of mites emerging from each bucket, divided by the starting number of mites, represents the reproductive success of a mite in the burial chamber. The pattern of emergence, i.e. the numbers of mites emerging on each dispersal vector (the male and female parents and the offspring) can also provide valuable information on relative survivorship during dispersal on beetles flying from the burial site.

KBS mites which had chosen N. orbicollis or N. tomentosus were raised with these two species beginning on August 8 and September 8, 1986, respectively. This experiment was a 2 x 2 factorial design, crossing mite behavioral phenotypes (TYPE effect) with beetle species (HOST effect), using 15-18 beetle pairs per treatment. The two beetle species treatments were run at different times since the beetle

species reproduce during different seasons. Differences between treatments were analyzed by ANOVA with planned contrasts performed between HOST treatments within TYPE, and between TYPE treatments within HOST. UMBS mites taken from N. defodiens were raised with N. defodiens and N. orbicollis beginning on June 2, 1987 and UMBS mites from N. tomentosus were raised with that species beginning on Sept. 11, 1987. Mites and N. sayi from UMBS and KBS were tested beginning on May 10, 1988. 15-18 beetle pairs were raised for each treatment. Differences between UMBS treatments in numbers of offspring were analyzed using analysis of variance, with planned contrasts performed between the preferred and non-preferred beetle species.

The behavioral and morphological experiments reported in Chapter 2 showed that orbicollis-specialists were not found at UMBS, despite the presence of this beetle species. One hypothesis explaining this distribution is that fitness of mites in association with UMBS beetles is too low to allow populations of the orbicollis-specialist to be maintained. I tested the prediction that UMBS N. orbicollis should support lower reproductive success than KBS beetles by raising KBS orbicollis-specialist on 6 pairs of beetles from each site, beginning on August 6, 1988. The standard protocol for reproductive success experiments was used (see above).

II. Generation time experiments

The protocol for setting up these experiments was identical to the reproductive success experiments above, except that beetles and mites were raised in 4 liter plastic shoe boxes instead of buckets. The boxes were filled with potting soil instead of forest soil. These

experiments were run only with KBS orbicollis- and tomentosus-specialists raised on both N. tomentosus and N. orbicollis. 40 N. orbicollis pairs (20 for each treatment) were initiated on 15 August 1985 and 42 N. tomentosus pairs (21 for each treatment) on 4 September 1985. Every 1-2 days after beginning each experiment I sampled 3 boxes at random from each treatment by removing the carcass and surrounding soil and preserving them in alcohol. The mites were later extracted from the soil using the following methods: The soil and carcass were suspended in 3.0 liter of tap water and washed through No. 25 and No. 400 sieves. The contents of the latter sieve were transferred to a 50 ml centrifuge tube and suspended in water. The contents were centrifuged at 2000 rpm for 3 minutes, and the water was poured off. The sediment was then suspended in 1.17 SG sucrose solution and centrifuged at 2000 rpm for 1 minute. The supernate was poured off into a No. 400 sieve, the mites washed with distilled water and then transferred to 70% ethanol. The numbers of each developmental stage in each sample were counted under a stereo microscope.

RESULTS

I. Reproductive success experiments --

Results of experiments using UMBS beetles and mites indicate that mites have higher reproductive success in association with their preferred beetle species (Table 3.1). The two most highly preferred species, N. defodiens and N. tomentosus, supported significantly higher reproduction than the less preferred species, N. sayi and N. orbicollis (ANOVA with planned contrast, $p<.001$). The lowest mean

Table 3.1. Reproductive success experiments using UMBS beetles and mites.

	# of offspring (per capita)			Proportion of total number		
Beetle species	n	mean	s.d.	n	mean	s.d.
N. defodiens						
Male	15	14.8	9.0	15	0.29	0.12
Female	15	14.7	7.9	15	0.31	0.17
Offspring	15	19.6	9.1	15	0.40	0.11
TOTAL	**15**	**49.0**	**12.3**			
N. tomentosus						
Male	15	10.9	10.2	14	0.28	0.22
Female	14	23.0	13.4	14	0.70	0.22
Offspring	15	0.8	0.9	14	0.02	0.02
TOTAL	**14**	**33.0**	**14.2**			
N. sayi						
Male	14	12.6	12.9	14	0.50	0.26
Female	15	12.3	8.7	14	0.47	0.26
Offspring	15	1.2	2.0	14	0.03	0.05
TOTAL	**14**	**24.6**	**18.5**			
N. orbicollis						
Male	15	8.5	4.7	11	0.48	0.19
Female	13	7.9	2.9	11	0.48	0.18
Offspring	13	0.8	1.1	11	0.04	0.04
TOTAL	**11**	**17.4**	**7.0**			

number of offspring was produced in association with UMBS N. orbicollis. This is significant, given the extreme avoidance of this species by UMBS mites (Fig 2.3).

Results of experiments using KBS mites and beetles provide a less clear picture of the adaptive nature of specialization at this site (Table 3.2). The KBS tomentosus-specialist disperses 55% more offspring in association with its preferred than with its non-preferred beetle species. In contrast, there is no significant difference between the mean number of offspring produced by the orbicollis-specialist on the two beetle species (Table 3.3). Thus, in terms of reproductive success, the orbicollis-specialist does not suffer a loss in fitness when it reproduces with the "wrong" species, as does the tomentosus-specialist. The patterns of mite emergence from the burial chambers when in association with the two species are, however, strikingly different. This is reflected in the percentage of offspring emerging on each dispersal vector, the male and female parents and the offspring. All orbicollis-specialist offspring emerged on the female N. tomentosus, while orbicollis-specialist offspring raised on N. orbicollis emerged on males, females and offspring (Table 3.3). This difference in emergence pattern may result in differences in survivorship during dispersal, when the beetles take flight to disperse from the burial site (see Discussion).

These experiments also provide data on the amount of time parents of each beetle species remain in the burial chamber and provide parental care to their offspring (Figure 3.1). Most members of the same species and sex disperse over a 3-4 day period, with the

Table 3.2. Reproductive success experiments using KBS beetles and mites.

	Orbicollis-specialist						Tomentosus-specialist					
	# of offspring (per capita)			Proportion of total number			# of offspring (per capita)			Proportion of total number		
Beetle species	n	mean	s.d.	n	mean	s.d.	n	mean	s.d.	n	mean	s.d.
N. orbicollis												
Male	17	5.9	4.4	15	0.21	0.14	15	12.3	8.1	14	0.46	0.17
Female	17	12.4	7.1	15	0.47	0.19	15	8.3	5.9	14	0.33	0.16
Offspring	15	8.7	4.1	15	0.33	0.11	15	5.9	4.6	14	0.21	0.11
TOTAL	**15**	**27.1**	**8.1**				**14**	**27.1**	**14.7**			
N. tomentosus												
Male	16	0.0	0.0	16	0.00	0.00	18	3.5	6.2	17	0.06	0.12
Female	16	27.5	10.1	16	1.00	0.01	17	35.7	11.4	17	0.84	0.13
Offspring	16	0.1	0.3	16	0.00	0.01	18	4.0	2.4	17	0.09	0.05
TOTAL	**16**	**27.6**	**10.1**				**17**	**42.4**	**11.6**			

Table 3.3. Statistical tests of significant differences for planned contrasts in the KBS reproductive success experiments. [* -- $p < 0.05$; ** -- $p < 0.01$; *** -- $p < 0.001$]

 # on male

	O		T
		**	
No	5.9	-----	12.3
		|	|
	* |		| ***
		|	|
Nt	0.0	-----	0.0
		ns	

% on male

	O		T
		***	
No	0.21	-----	0.46
		|	|
	*** |		| ***
		|	|
Nt	0.00	-----	0.06
		**	

 # on female

	O		T
		ns	
No	12.4	-----	8.3
		|	|
	*** |		| ***
		|	|
Nt	27.5	-----	35.7
		*	

% on female

	O		T
		*	
No	0.47	-----	0.33
		|	|
	*** |		| ***
		|	|
Nt	1.00	-----	0.84
		***	

 # on offspring

	O		T
		*	
No	8.7	-----	5.9
		|	|
	*** |		| ns
		|	|
Nt	0.1	-----	4.0
		***	

% on offspring

	O		T
		***	
No	0.33	-----	0.21
		|	|
	*** |		| ***
		|	|
Nt	0.00	-----	0.09
		***	

Total # of offspring

	O		T
		ns	
No	27.1	-----	27.1
		|	|
	ns |		| ***
		|	|
	27.6	-----	42.4
		***	

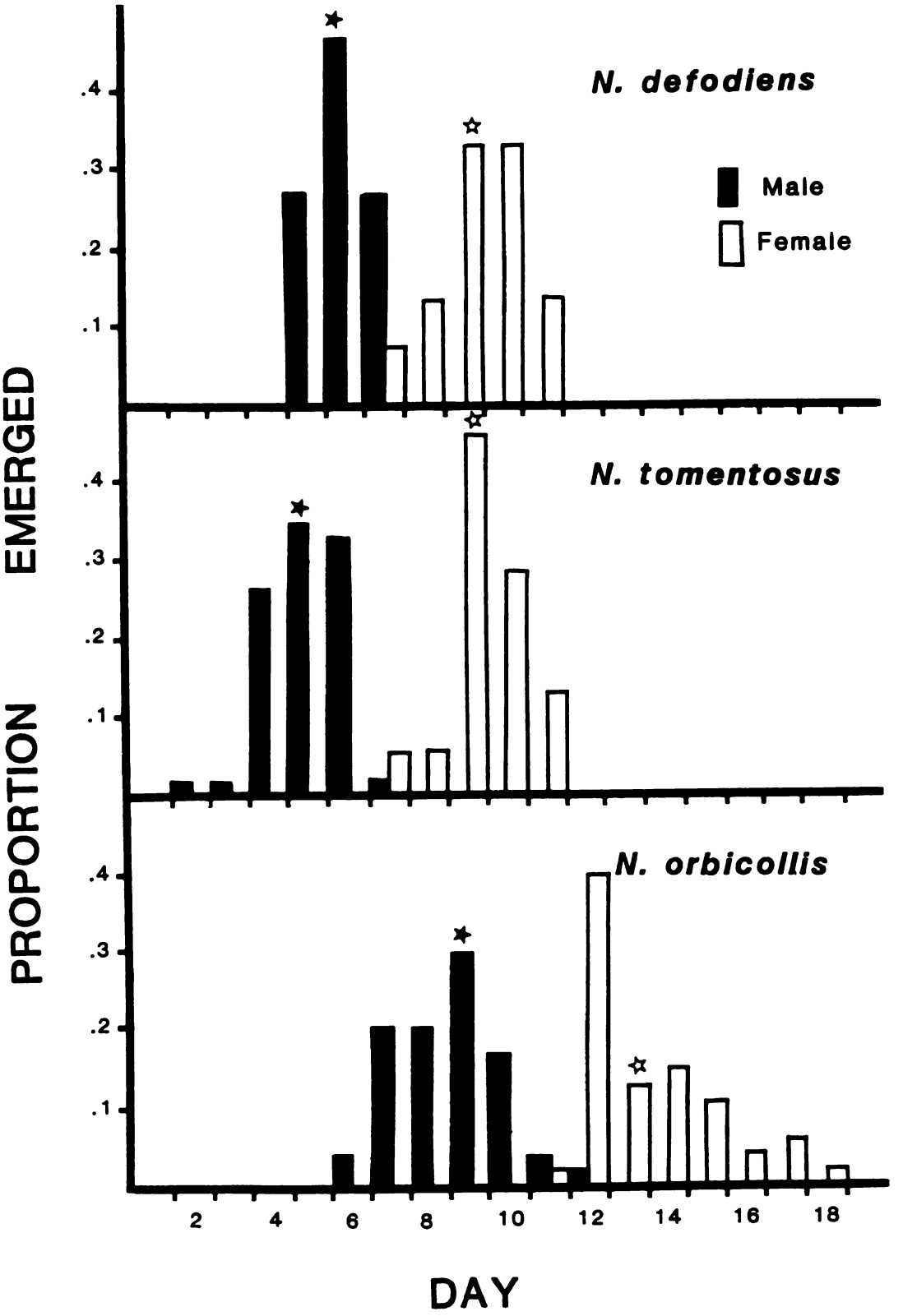


Figure 3.1 Parental dispersal for three species of Nicrophorus. The proportion of each sex which emerged is plotted against the day of emergence (i.e. number of days after the pair is placed on a mouse).

exception of N. sayi, who may not emerge for up to 40 days (not shown in Figure 3.1). Males disperse earlier than females in all species tested. N. orbicollis disperse 4-5 days later than N. defodiens and N. tomentosus; the latter two species are very similar in dispersal pattern. The importance of these differences between beetle species will become apparent when considering the generation times of the mite specialists (see Generation time experiments below and Discussion).

KBS orbicollis-specialists were raised with N. orbicollis from KBS and UMBS to test whether the lack of the orbicollis-specialist at UMBS could be due to low fitness in association with UMBS N. orbicollis. There was no significant difference between the number of mite offspring dispersed from beetles from the two sites. In fact, a non-significantly larger mean number of mites emerged from UMBS beetles (27.2 vs. 25.7, T-test, $p > 0.8$, d.f.=9) [The mean for the orbicollis-specialist on KBS beetles is also not different from the mean number of offspring emerged in the 1985 KBS trials (also the orbicollis-specialist with KBS N. orbicollis), indicating the repeatability of these experiments done 3 years apart, but under standardized conditions.] KBS and UMBS (1986 and 1987) reproductive success experiments also showed that N. orbicollis males from the two sites have the same median day of dispersal, i.e. day 9. In other words, the lack of a orbicollis-specialist mite at UMBS (Chapter 2) cannot be explained by a lower reproductive success in association with beetles at this site. It also cannot be explained by behavioral differences between N. orbicollis from the two sites in paternal care time, which may affect the dispersal component of fitness.

II. Generation time experiment

There were obvious differences in generation time between the two KBS behavioral types. Figure 3.2 shows developmental curves for the two types on both hosts. The orbicollis-specialists complete a generation cycle (deutonymph to deutonymph) in 8-11 days, while the tomentosus-specialists do so in 6-8 days. Raising mites on N. orbicollis results in slightly slower development than on N. tomentosus for both behavioral types, but this effect does not eliminate the differences between mite types.

The generation time of UMBS mites was not directly measured, but can be inferred from the dispersal patterns of the beetle species (Fig. 3.1) and the number of mites dispersed on each vector (Table 3.1). All male N. defodiens and N. tomentosus carried some deutonymphs, a few as early as day 5. This suggests a generation time for UMBS mites similar to that of the KBS tomentosus-specialist.

DISCUSSION

The goal of these experiments was to ascertain whether the mites' biased use of beetle-species at the two sites was adaptive, i.e. do mites achieve higher fitness when in association with their preferred beetle species? At UMBS, the most highly preferred beetle species, N. tomentosus and N. defodiens, support higher reproductive success than the less preferred species, N. sayi and N. orbicollis (Table 3.1). UMBS mites also seem to switch their preference to whichever beetle species is reproducing at the time (Figure 2.2, Chapter 2). The switch between preference for N. tomentosus and N. defodiens is

Figure 3.2. Rates of development for KBS mites. Shown are means (2-3 samples) of the percent of each lifestage in the sample for both specialists (orbicollis- and tomentosus-) on both hosts. The white fields are percent larvae (L), the striped fields percent protonymphs (P), and the black fields percent deutonymphs. Day refers to the number of days after the beetle pair was placed on a mouse.

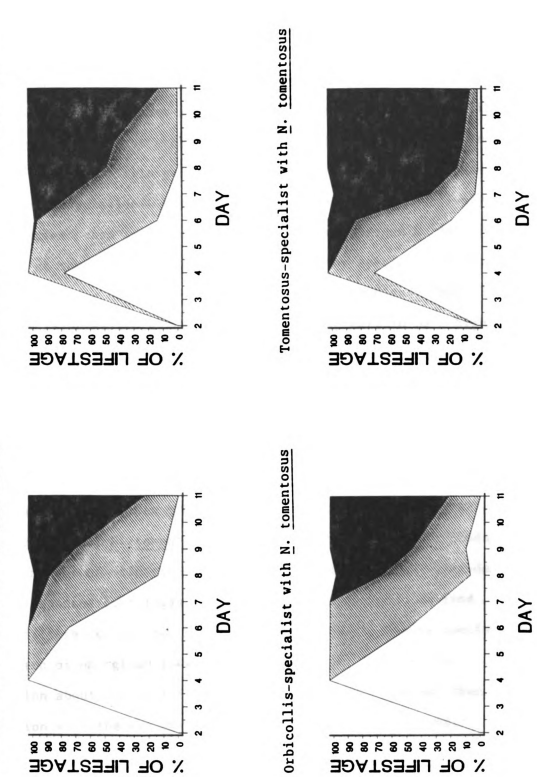

Orbicollis-specialist with N. orbicollis
% OF LIFESTAGE
DAY
Tomentosus-specialist with N. orbicollis
% OF LIFESTAGE
DAY
Orbicollis-specialist with N. tomentosus
% OF LIFESTAGE
DAY
Tomentosus-specialist with N. tomentosus
% OF LIFESTAGE
DAY

Figure 3.2

associated with breeding season, and the heightened attractiveness of N. sayi in September suggests that mites may be switching onto N. sayi in fall in preparation for their early spring reproductive period (Figure 2.2). The ability to switch from non-reproductive to reproductive beetle species should be highly favored, as long as the cost of switching is not prohibitive. A behavior which results in the addition of even a single generation over the season should spread quickly in a population. Although the mites prefer reproductively active species, when two species (i.e. N. orbicollis and N. defodiens) reproduce at the same time, P. carabi prefers the species with which it experiences the higher reproduction (i.e. N. defodiens; Table 3.1). UMBS mites' flexibility of beetle-species-use appears to be a highly adaptive strategy, assuring the highest possible reproduction over the largest number of generations possible in a year.

The KBS results do suggest that habitat selection is adaptive if only reproductive success is considered. While the tomentosus-specialist has a higher number of offspring with its preferred species, N. tomentosus, the orbicollis-specialist produces the same number of offspring with either species. One must consider another component of fitness, survival during dispersal, to find fitness differences between association with the two beetle species. The pattern of emergence from the burial chambers can provide information about the relative survivorship during dispersal when in association with the two beetle species. Orbicollis-specialist offspring emerge on all possible vectors (male and female beetle parents and offspring) when in association with N. orbicollis. But when the same mite type is raised with N. tomentosus, all offspring

(an average of 400 individuals) emerge on the female parent beetle (Table 3.2). A flying beetle rarely carries more than 50 mites (pers. obs.), presumably due to a limited number of "safe spots" on the beetle. In glasshouse trials, Springett (1968) found that beetles emerging from the burial chambers carried several hundred mites, but carried fewer than 10% of their original load when allowed to fly for 5 minutes. When raised with N. orbicollis, orbicollis-specialist offspring are spread over all possible dispersal vectors, filling the "safe spots" on several beetles, rather than on a single female, as occurs when in association with N. tomentosus. Thus, despite no difference in reproductive success when in association with the two beetle species, the difference in emergence patterns assures that more offspring will survive dispersal on N. orbicollis than N. tomentosus.

But what kind of differences between beetle species result in fitness differences for the mites? Differences in reproductive success cannot be explained directly by my experiments. Beetle species do differ significantly in how they bury carcasses, specifically in burial depth (Wilson, 1986). These differences may affect mite fecundity and survival in the burial chamber by resulting in differences in temperature, relative humidity or competition or predation from other organisms. One aspect of fitness, survivorship during dispersal, which I have argued can be inferred from the pattern of mite emergence from burial chambers, is strongly affected by synchronization between the mites' generation time and beetles parental care behavior. The generation times of the KBS mite types are synchronized with the median day of departure of the male parent of their preferred species, i.e. their first opportunity to disperse.

This synchronization breaks down when mites are raised with their non-preferred beetle species. Orbicollis-specialists, for example, complete a generation and are ready to disperse after 8-10 days (Figure 3.2). By this time N. tomentosus males have already dispersed (Figure 3.1). Long generation time results in missed dispersal opportunity. Tomentosus-specialists, in contrast, are ready to disperse after 6 days (Figure 3.2) but may wait several days before dispersing with N. orbicollis males (Figure 3.1). This waiting period may be another explanation for the lower reproductive success of tomentosus-specialists when raised in association with N. orbicollis, if mites which embark on a male beetle which is still engaging in parental behaviors suffer mortality. These considerations suggest that the two beetle species represent habitats which differ in their optima for mite generation time. Synchronization of generation time with male dispersal in one beetle species results in a fitness tradeoff in terms of reproductive success or survivorship during dispersal when reproducing with the other available beetle species. These types of tradeoffs in fitness are expected if specialization is to evolve or be maintained in either an inter- or intraspecific setting.

The pattern of mite and beetle species compositions seen at the two study sites is somewhat counter-intuitive. As one moves north from KBS, the beetle community is augmented by the presence of N. defodiens. The mite community at UMBS, however, lacks an N. orbicollis specialist, despite the presence of this beetle species. This geographical pattern does not follow the predictions of a standard host-parasite model, which is often assumed when considering phoretic

mite host associations. Hunter and Rosario (1988), for example, propose that close mite-host association probably evolved in phoretic mites as a result of niche specialization by host species, during which time mites adapted to a particular host and become reproductively isolated from mites on other hosts. This scenario does not predict well the pattern seen in P. carabi. In this case, a close association with one host disappears when an additional host becomes available.

The observed changes in the mites' degree of specialization is correlated with change in the beetle species community. This seems to reflect more closely the behavior of a consumer-resource rather than host-parasite model. Let us consider such a scenario: a non-specialist mite is initially common on all Nicrophorus species. It reproduces successfully in association with all species and generally uses species in proportion to their frequency (i.e. there is no preference). However, since beetle species differ in characters which affect the fitness of mites, different mite phenotypes may be more fit in association with certain beetle species. In other words, the beetle species represent different habitats from a mites point of view. If fitness differences are large enough, selection should favor biased use of beetles, i.e. habitat preference. Preference appears to be a evolutionarily labile trait, as evidenced by its geographical variation (see Chapter 5). One may expect, therefore, that specialization in beetle species use should be sensitive to local selection pressures. For example, if a beetle species which allowed relatively higher reproduction of mites invaded a community in which many beetle species were used, one might expect a shift toward

specialization on this species in response to natural selection. A mite's perspective of the available beetle species is similar to an herbivorous insect's view of a forest or grassland: the problem is not how to locate a resource, but how and whether to discriminate between types of resources in order to pick the ones which support the relatively highest survival and reproduction.

In addition to differences in selection in two habitats, the presence of habitat specialists can be influenced further by two important factors, mating structure and competition. Since mites reproduce in association with the beetles, strong habitat preferences results in biased mating between members of a species with similar habitat preference. Thus, if a single species is faced with two habitats, the result under certain conditions of selection between habitats is the evolution of an intraspecific polymorphism for habitat specialization. Whether this situation leads to speciation depends strongly on the degree to which assortative mating is a correlated character of habitat selection (i.e. the degree to which mating occurs in the habitat) (Chapter 4; Rice, 1987; Diehl and Bush, 1989). Competition can also effect the presence of habitat specialists through the action of density-dependent fitness functions within habitats (Rosenzweig, 1979, 1981; Pimm and Rosenzweig, 1981). For example, a habitat to which an organism is not adapted in terms of some optimal value of a fitness trait may be used if density-dependence drives down fitness in the "preferred" habitat. The use of the non-preferred habitat may result in the exclusion of the specialist for that habitat under certain conditions. These factors play an important role in two evolutionary scenarios which I

have developed to explain the distribution of habitat specialists at my two study sites in Michigan.

A local adaptation scenario

The interaction between habitat selection and population structure could explain the differences in mite species composition at the two sites. At KBS, in order for mites to reproduce from June through September, they must use two species which differ significantly in their parental leaving times. I have argued that these two species represent to the mites two distinct habitats, in which different trait values are most fit. A single population using both these species should experience disruptive selection on generation time and other characters involved in tradeoffs in fitness between association with the two beetle species. In addition, mites which prefer N. orbicollis are in part spatially and seasonally isolated from mites which prefer N. tomentosus, due to the seasonal differences in reproduction of these two beetle species (Figure 1.2). The expectation is that two distinct specialist species would evolve at KBS.

At UMBS, on the other hand, a beetle species not found at KBS, N. defodiens, is very numerous. N. defodiens is not significantly different than N. tomentosus in parental leaving times (Figure 3.1) and reproduces during the same period seasonally as N. orbicollis. Thus, with respect to generation time at least, N. defodiens and N. tomentosus present a single selective regime from June through September. N. orbicollis also commonly take over carcasses from N. defodiens (Wilson, et. al. 1984, Trumbo, unpubl.), so that preference for one species does not always put a mite in a predictable selective

environment nor result in biased mating between mites with the same preference. Disruptive selection is not associated with predictable habitats or seasons, and assortative mating is not a strongly correlated character of habitat selection. The required conditions for speciation are not present. Instead of divergence into distinct specialist species, UMBS mites become seasonal specialists, choosing one beetle species at each season. This model predicts that a species would exhibit what might be called "patchy divergence," i.e. speciation by habitat specialization only in areas where local selection and mating structure allow it.

If the patchy divergence model is correct, one would expect the taxonomy of this group to be problematic. This is indeed the case. The more recent taxonomic treatments of the genus (e.g. Hyatt, 1980) fault earlier workers for relying on characters which vary "within samples." The result of these observations is a current tendency to subsume many named species in this genus into a single species, P. carabi (Micherdzinski, 1969; Hyatt, 1980; Korn, 1983). My studies suggest that "variation within a sample" may in fact reflect the existence of closely related specialist species (e.g. at KBS). This variation, however, may not be found everywhere. Under a patchy divergence model, morphological differentiation may proceed on different characters in different locations, or intraspecific morphological variation at one site may show up interspecifically at another. I know of no taxonomic treatment which takes into account host associations or mite behavior and suggest that this is crucial for a satisfactory treatment of this genus.

A local competition scenario

Despite the apparent congruence of the local divergence hypothesis to the current pattern of host use in P. carabi, an alternative hypothesis involving a different model divergence between the specialists at KBS should be considered. Specifically, one should consider the evolutionary history of the host species, since the mite specialists at KBS may have diverged when the beetles species involved, N. orbicollis and N. tomentosus, were allopatrically distributed. A phylogenetic classification of the New World Nicrophorus by Peck and Anderson (1985) places 13 of the 15 species into four species groups. N. orbicollis is considered the northernmost member of a group of primarily Central and South American species, the so-called "orbicollis-group." This group may be the earliest species group to enter North America from Eurasia, the presumed center of origin for the genus. N. defodiens and tomentosus are placed in two separate species groups, each of which is thought to represent one or more ancestral invasions of North America from Eurasia via land bridges during the Tertiary or Pleistocene. P. carabi's use of both N. defodiens and N. tomentosus (not closely related species) at UMBS suggests the lack of close mite-beetle coevolution. However the association of the orbicollis-specialist with N. orbicollis suggests that this mite may represent the descendants of mites which crossed to North America with the orbicollis-group ancestor and thus speciated from later invading mites in allopatry. While this hypothesis is consistent with the behavioral and life history differences between orbicollis- and tomentosus-specialists at KBS, it does not explain why the

orbicollis-specialist is not found at UMBS. As I have shown in this chapter, KBS orbicollis-specialists do not suffer decreased reproductive success when raised with UMBS N. orbicollis. A difference in the nature of this beetle species at UMBS cannot explain the mite's absence. Rather there must be something different about the environment, in the broadest sense, at UMBS.

I suggest there are two possible differences in environment between the two sites. First, there could be an unknown abiotic factor, such as temperature, which limits the orbicollis-specialist's northerly distribution someplace to the south of UMBS. A better understanding of the overall distribution of the orbicollis-specialist and how it relates to abiotic factors could support this explanation. Second, the difference between sites could be in the competitive environment which the orbicollis-specialist experiences. At KBS, the preference of the tomentosus-specialist for N. tomentosus means that the orbicollis-specialist competes only with mites which have reproduced on that beetle species. At UMBS, mites from N. tomentosus and N. defodiens avoid N. orbicollis when given a choice, but the frequency with which N. orbicollis individuals take over carcasses buried by N. defodiens means that mites are constantly shunted from their preferred 'habitats,' the beetle species N. tomentosus and N. defodiens, to reproduction in association with N. orbicollis. If the presence of UMBS (or tomentosus-specialist) mites has a significant density-dependent effect on the fitness of orbicollis-specialist mites, the unidirectional shift of mites from N. defodiens to N. orbicollis could result in a significant drop in the fitness of the orbicollis-specialists. The effect of this process on the ability of

the orbicollis-specialists to maintain viable populations depends on the number of mites shifted from N. defodiens to N. orbicollis, the competitive equivalence of the two mite specialists, and the shape of the fitness vs. density curve on N. orbicollis. This interspecific competition scenario is consistent with the pattern of beetle species use between the two sites.

CHAPTER 4. A SIMULATION MODEL OF THE EFFECT OF MIGRATION ON LOCAL ADAPTATION AND DIVERGENCE

In the previous chapter, I proposed a model of local speciation by habitat specialization to explain the variation of morphological, behavioral and life history patterns I observed between my two main study sites in Michigan. I proposed that conditions of selection and population structure favoring speciation between mites using the two beetle species N. orbicollis and N. tomentosus were present at the southern site, KBS. At the northern site, UMBS, the presence of the beetle species N. defodiens renders conditions insufficient for the evolution of reproductive isolation between habitat specialists. I proposed that an alternative, local solution to host (beetle species) use by these mites had evolved, i.e. seasonal shifts in beetle species preference.

While this explanation is consistent with the observed patterns it makes an implicit assumption that the conditions at each site are a result of recent adaptive evolution. It ignores the effects that migration may play in altering patterns of species composition at the two sites. Consideration of migration raises one question in particular about this system: If the KBS orbicollis- and tomentosus-specialists are reproductively isolated species, as my data indicate, what prevents that orbicollis-specialist from migrating northwards to UMBS? Its preferred beetle species, N. orbicollis, is a dominant member of the beetle community at UMBS, and might be considered an 'empty niche' there, unused as a carrier for P. carabi. At first glance, there seem to be two answers to this question (which

are not necessarily mutually exclusive). First, the evolution of the two specialists into reproductively isolated species may be a recent occurrence; there simply has not been sufficient time for the orbicollis-specialist to spread north to UMBS. Second, there may be something about the mechanisms of local adaptive evolution of habitat use which slows or prevents the spread of habitat specialists (including distinct species) to areas in which conditions are insufficient for in situ population divergence. In this chapter I present a general model of local speciation by habitat specialization, and outline the conditions which could slow or prevent the spread of newly evolved specialists to areas away from their centers of origin. I will first consider a one-population model of speciation, patterned after models of Felsenstein (1981) and Diehl and Bush (1989), which demonstrates the conditions which promote local speciation based on habitat selection. My goal was to build a model which covers the range of conditions contained in the above two models. Differences between my model and the models above are noted in the text below. I then expand the model to multiple populations in order to directly address the spread of newly-evolved habitat specialists.

I. Single population model

METHODS

Most models investigating the maintenance of polymorphisms in single populations and the evolution of sympatric divergence trace their roots back to an analytical model of Levene (1953; see reviews of modelling history in Hedrick et al. 1976 and Hedrick, 1986). Levene's model proposes that a single population may occupy 2 discrete habitats, with population size independently regulated in each

habitat. This situation is referred to as soft selection, and actually represents a form of frequency-dependent selection. My model follows these principles, following the frequencies of all genotypes in the populations. Genotypes are haploid and have three unlinked loci, A, B and C, each with two alleles. The first locus, A, affects habitat selection; the second, B, affects fitness in the two habitats; and the third locus, C, affects assortative mating between genotypes. Alleles have differential effects as described below. Reproduction occurs through the union of haploids, followed by recombination and production of haploid individuals. The life cycle (i.e. one generation) consists of five stages, described below as habitat selection, selection within habitats, population regulation, pre-mating migration, and mating (Figure 4.1). Figure 4.2 summarizes the life cycles in Felsenstein's and Bush and Diehl's models.

Habitat selection. The first locus, A, affects habitat selection, such that individuals with the A_1 allele move preferentially to Habitat 1 and those with allele A_2 to Habitat 2. The penetrance of the habitat selection locus, p_A, affects habitat use in the following manner:

$$Freq_1(A_1) = Freq_{pop}(A_1) * (1+p_A)/2$$
$$Freq_2(A_1) = Freq_{pop}(A_1) * (1-p_A)/2$$
$$Freq_1(A_2) = Freq_{pop}(A_2) * (1-p_A)/2$$
$$Freq_2(A_2) = Freq_{pop}(A_2) * (1+p_A)/2,$$

where $Freq_{pop}(A_j)$ and $Freq_i(A_j)$ are the frequencies of all genotypes with allele A_j in the entire population and in habitat i, respectively. Alternatively, penetrance can be thought of as the correlation between

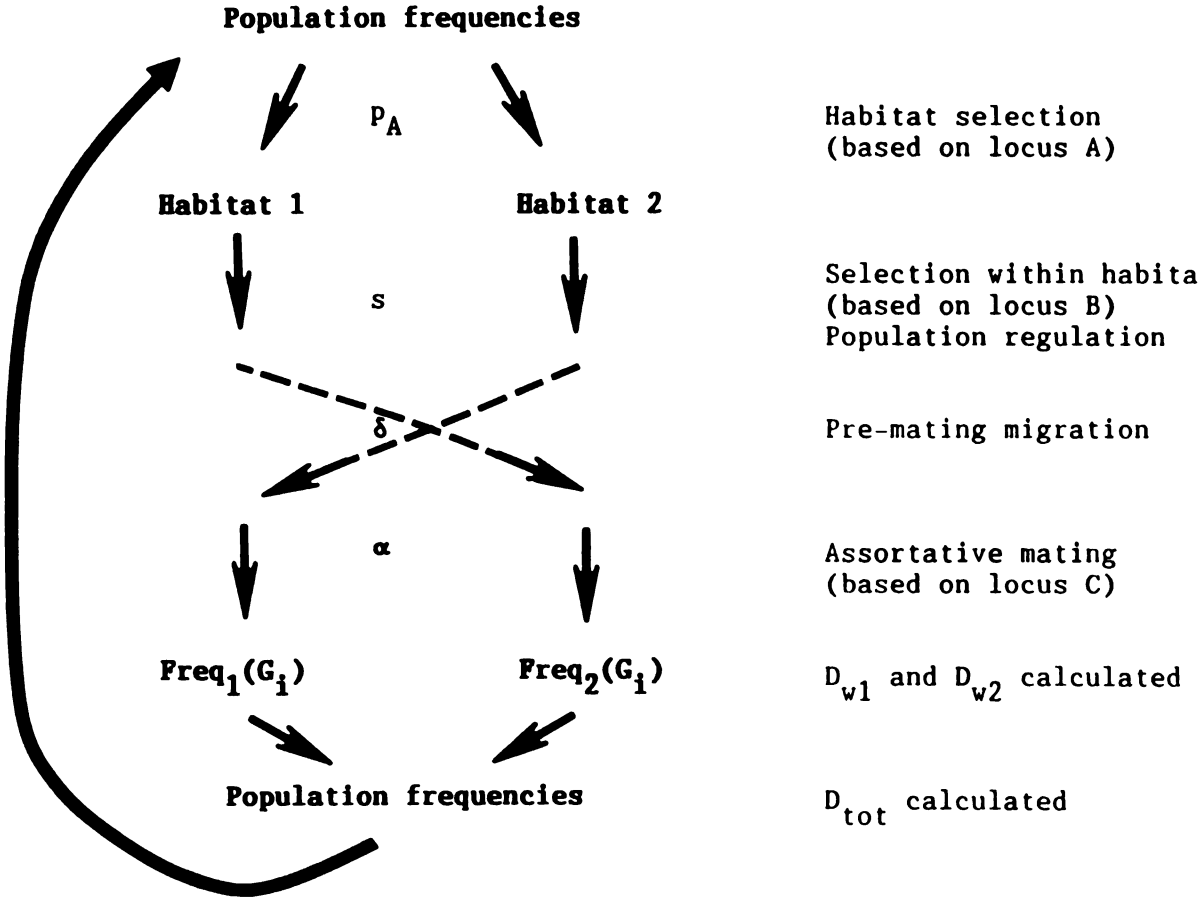


Figure 4.1 Life cycles and associated parameters for one-population model.

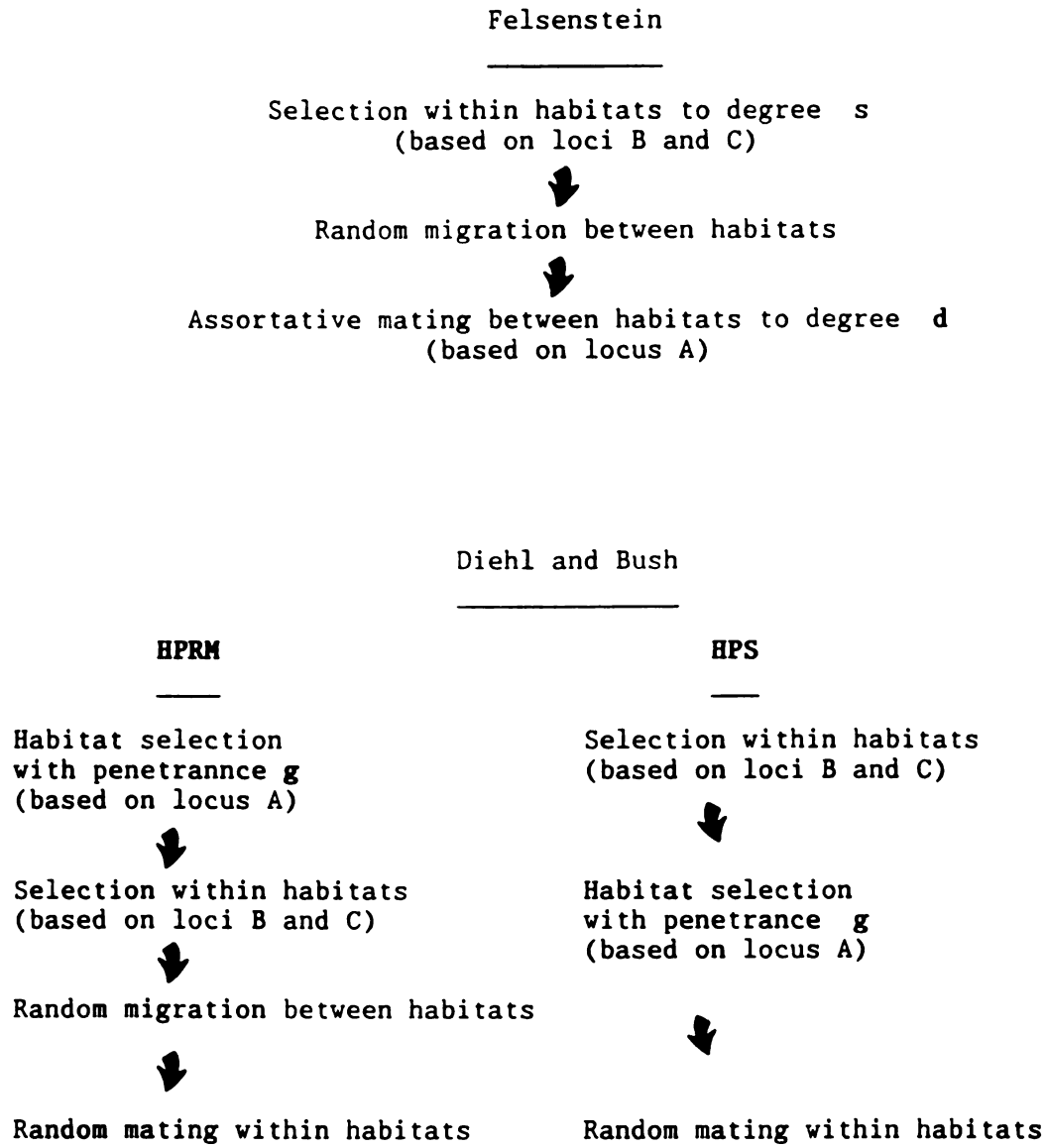


Figure 4.2 Life cycles and parameters for models of Felsenstein (1981) and Diehl and Bush (1989). HPRM and HPS refer to Bush and Diehl's Habitat Preference with Random Mating and Habitat Preference Speciation models, respectively.

an individual's habitat preference and the habitat it actually ends up in (i.e. the predictability of habitat selection). I varied the penetrance of the habitat selection locus (p_A) from 0.0 (no habitat selection) to 0.99 (almost perfect habitat selection), with intermediate values set at 0.2, 0.5 and 0.8. In Felsenstein's model, individuals may reside in one of two habitats but move randomly between habitats each generation. In Diehl and Bush's model, habitat selection occurs either before or after natural selection, and is controlled by locus A. When habitats are sympatric, their parameter g is identical to p_A (Figures 4.1 and 4.2).

Selection within habitats. Once individuals move to habitats, natural selection acts on individuals based on the second locus, B, with the B_1 allele favored in Habitat 1 and the B_2 allele favored in Habitat 2. The strength of selection is indicated by the parameter s, which influences frequencies in the habitats in the following manner:

$$Freq_i'(B_i) = Freq_i(B_i) * (1+s) \text{ for } i=1 \text{ and } 2,$$

where $Freq_i(B_i)$ is the frequency of genotypes with allele B_i. I varied the strength of habitat-based selection, s, between 0.0 and 1.0, with intermediate values at 0.1, 0.5 and 0.8. In both Felsenstein's and Diehl and Bush's models, selection was based on two loci which were allowed to interact either additively or multiplicatively. Due the number of other loci in this model, I chose to base selective differences between habitats on one locus only in order to keep the recursion equations as simple as possible.

Population regulation. After selection within habitats occurs on

the basis on locus A, the frequencies of genotypes in each habitat are corrected to sum to 1.0. This simulates density-dependent control of habitat population size, with each habitat regulated independently. Habitats are assumed in all simulations to be of equal size.

Pre-mating migration. In this step of the life cycle a proportion of each habitat's population is shifted to the opposite habitat. This step precedes mating within the habitat, and serves as a method of varying the degree to which mating occurs in the habitat (or the degree to which assortative mating is a correlated character of habitat selection (Rice, 1987)). Genotype frequencies are adjusted in the following manner:

$$Freq_1'(G_j) = (1 - \delta) * Freq_1(G_j) + \delta * Freq_2(G_j)$$
$$Freq_2'(G_j) = (1 - \delta) * Freq_2(G_j) + \delta * Freq_1(G_j),$$

where $Freq_i(G_j)$ is the frequency of the 3-locus genotype j in habitat i. δ describes the degree of habitat-based mating and varies between 0.5 (random mating) and 0.0 (complete habitat-based mating). These two extremes reflect conditions in Felsenstein's and Bush and Diehl's models. Intermediate levels of δ (here set at 0.1, 0.25 and 0.40) reflect natural situations in which mating may occur near habitats or hosts, but they are closely interspersed (fine-grained), or when mating occurs at a few centralized locations and habitats are clumped (coarse-grained).

Mating. Mating occurs randomly within habitats with respect to the A and B loci, but assortatively at the C locus. Assortative mating occurs at locus C to degree, α, according to the following rules:

Genotypes mate with other genotypes carrying the same C-allele (C_1 or C_2) with frequency α, and randomly with all genotypes with frequency $(1-\alpha)$. This method of assortative mating is identical to Felsenstein's method (with α equivalent to his parameter d) and does not result in changes in allele frequencies due to mating alone. Assortative mating based on a genetic mating type does not occur in Bush and Diehl's models. Since 8 genotypes are possible, yet assortative mating occurs at only one locus (i.e. C), the recursion equations for genotypes are complex and are given in Table 4.1. I varied α from 0.0 (no assortative mating) to .99 (almost perfect assortative mating) in the simulations. Intermediate values were set at 0.2, 0.5 and 0.8.

Population frequencies recovered. The population wide frequencies of all genotypes are calculated by averaging the two within-habitat genotype frequencies, since in these simulations, habitats are assumed to be of equal size. The life cycle is then started again with the assortment of genotypes into habitats (i.e. habitat selection). These sorts of life cycles are realistic for many species of herbivores, parasites, and phoretic associates, which themselves represent a large proportion of biological species (Bush, 1975), as well as other organisms which are habitat specific.

The description of variables and their ranges in the simulations are given in Table 4.2. Starting conditions were with all "2-alleles" (i.e. A_2, B_2, and C_2) at mutation frequencies of .001 and all population genotypic frequencies at Hardy-Weinberg equilibrium. Simulations were run until the frequency of the $A_1B_1C_1$ genotype changed less than .00001 in 100 generations. At that point the level of

Table 4.1. Recursion equations for mating of genotypes within habitats. [f_{111} = Freq($A_1B_1C_1$), etc.]

$$\text{Freq}'(A_1B_1C_1) = \left[1 - \alpha + \alpha/(f_{111} + f_{121} + f_{211} + f_{221})\right] *$$
$$\left[f_{111} * (f_{111} + f_{121} + f_{211} + f_{221}/2) + f_{121} * f_{211}/2\right] +$$
$$(1 - \alpha) * \left[f_{111} * (f_{112} + f_{122}/2 + f_{212}/2 + f_{222}/4) +\right.$$
$$\left. f_{112} * (f_{121}/2 + f_{211}/2 + f_{221}/4) + f_{121} * f_{212}/4 + f_{122} * f_{211}/4\right]$$

$$\text{Freq}'(A_1B_1C_2) = \left[1 - \alpha + \alpha/(f_{112} + f_{112} + f_{212} + f_{222})\right] *$$
$$\left[f_{112} * (f_{112} + f_{122} + f_{212} + f_{222}/2) + f_{122} * f_{212}/2\right] +$$
$$(1 - \alpha) * \left[f_{111} * (f_{112} + f_{122}/2 + f_{212}/2 + f_{222}/4) +\right.$$
$$\left. f_{112} * (f_{121}/2 + f_{211}/2 + f_{221}/4) + f_{121} * f_{212}/4 + f_{122} * f_{211}/4\right]$$

$$\text{Freq}'(A_1B_2C_1) = \left[1 - \alpha + \alpha/(f_{111} + f_{121} + f_{211} + f_{221})\right] *$$
$$\left[f_{121} * (f_{111} + f_{121} + f_{211}/2 + f_{221}) + f_{111} * f_{221}/2\right] +$$
$$(1 - \alpha) * \left[f_{121} * (f_{112}/2 + f_{122} + f_{212}/4 + f_{222}/2) +\right.$$
$$\left. f_{122} * (f_{111}/2 + f_{211}/4 + f_{221}/2) + f_{111} * f_{222}/4 + f_{112} * f_{221}/4\right]$$

$$\text{Freq}'(A_1B_2C_2) = \left[1 - \alpha + \alpha/(f_{112} + f_{112} + f_{212} + f_{222})\right] *$$
$$\left[f_{122} * (f_{112} + f_{122} + f_{212}/2 + f_{222}) + f_{112} * f_{222}/2\right] +$$
$$(1 - \alpha) * \left[f_{121} * (f_{112}/2 + f_{122} + f_{212}/4 + f_{222}/2) +\right.$$
$$\left. f_{122} * (f_{111}/2 + f_{211}/4 + f_{221}/2) + f_{111} * f_{222}/4 + f_{112} * f_{221}/4\right]$$

Table 4.1 (continued).

$$\text{Freq}'(A_2B_1C_1) = \left[1 - \alpha + \alpha/(f_{111} + f_{121} + f_{211} + f_{221})\right] *$$
$$\left[f_{211} * (f_{111} + f_{121}/2 + f_{211} + f_{221}) + f_{111} * f_{221}/2\right] +$$
$$(1 - \alpha) * \left[f_{212} * (f_{111}/2 + f_{121}/4 + f_{211} + f_{221}/2) +\right.$$
$$\left. f_{211} * (f_{112}/2 + f_{122}/4 + f_{222}/2) + f_{111} * f_{222}/4 + f_{112} * f_{221}/4\right]$$

$$\text{Freq}'(A_2B_1C_2) = \left[1 - \alpha + \alpha/(f_{112} + f_{112} + f_{212} + f_{222})\right] *$$
$$\left[f_{212} * (f_{112} + f_{122}/2 + f_{212} + f_{222}) + f_{112} * f_{222}/2\right] +$$
$$(1 - \alpha) * \left[f_{212} * (f_{111}/2 + f_{121}/4 + f_{211} + f_{221}/2) +\right.$$
$$\left. f_{211} * (f_{112}/2 + f_{122}/4 + f_{222}/2) + f_{111} * f_{222}/4 + f_{112} * f_{221}/4\right]$$

$$\text{Freq}'(A_2B_2C_1) = \left[1 - \alpha + \alpha/(f_{111} + f_{121} + f_{211} + f_{221})\right] *$$
$$\left[f_{221} * (f_{111}/2 + f_{121} + f_{211} + f_{221}) + f_{121} * f_{211}/2\right] +$$
$$(1 - \alpha) * \left[f_{221} * (f_{112}/4 + f_{122}/2 + f_{212}/2 + f_{222}) +\right.$$
$$\left. f_{222} * (f_{111}/4 + f_{121}/2 + f_{211}/2) + f_{121} * f_{212}/4 + f_{122} * f_{211}/4\right]$$

$$\text{Freq}'(A_2B_2C_2) = \left[1 - \alpha + \alpha/(f_{112} + f_{112} + f_{212} + f_{222})\right] *$$
$$\left[f_{222} * (f_{112}/2 + f_{122} + f_{212} + f_{222}) + f_{122} * f_{212}/2\right] +$$
$$(1 - \alpha) * \left[f_{221} * (f_{112}/4 + f_{122}/2 + f_{212}/2 + f_{222}) +\right.$$
$$\left. f_{222} * (f_{111}/4 + f_{121}/2 + f_{211}/2) + f_{121} * f_{212}/4 + f_{122} * f_{211}/4\right]$$

Table 4.2. Parameters and their ranges in the one population model.

Symbol	Range	Description
p_A	0.0 - 0.99	Penetrance of habitat selection locus (A)
s	0.0 - 1.00	Selection differential (on locus B)
δ	0.5 - 0.00	Degree of mating in the habitat
α	0.0 - 0.99	Degree of assortative mating (at locus C)

disequilibrium between the A and B loci was calculated. The total disequilibrium based on the entire population, D_{tot}, was partitioned into the disequilibrium due to differences between populations in genotype frequencies (D_{bet}), and the disequilibrium within populations (D_{w1} and D_{w2}). This was done by calculating D_{w1} and D_{w2} before subpopulations in the 2 habitats were combined to get overall population genotype frequencies. D_{bet} was then calculated as $D_{bet} = D_{tot} - (D_{w1} + D_{w2})/2$. (Nei and Li, 1973).

RESULTS

The results of the simulation can be divided into 6 categories of outcomes, according to which sets of "2-alleles" evolve to equilibrium:

1. No evolution. None of the 2-alleles evolve when there is no selective differential ($s = 0$) and the habitat selection locus has zero penetrance ($p_A = 0$). There is no disequilibrium between loci A and B. This is the trivial case where there is no difference between alleles at the two loci, thus the alternative "2-alleles" could only increase in frequency by drift, which is not a part of this model.

2. Differential habitat selection evolves. The habitat selection locus (A) evolves alone when $p_A > 0.0$ and $s = 0$. At equilibrium, $Freq(A_1B_1C_1) \approx Freq(A_2B_1C_1) \approx 0.5$; the number of generations required to reach equilibrium, G, is negatively related to p_A (G = 500 to 200, for $p_A = 0.2$ to 0.99, unless $\alpha = 0.99$, in which case $G \approx 1000$). There is no disequilibrium between loci A and B. This case demonstrates the frequency-dependent nature of these "Levene-models." The low

frequency behavioral type is always favored when penetrance is greater than zero. If one thinks in terms of individuals rather than genotype frequencies, the habitat favored by the minority behavioral type will always have fewer individuals in it, and thus each individual in this habitat is less affected by population regulation than the individuals in the more "popular" habitat. Thus the true selective differential between genotypes is not a constant, as is s, but a variable dependent on the frequency of the various genotypes in each habitat.

3. Differential habitat fitness evolves. The habitat fitness locus (B) evolves alone if $s > 0$ and $p_A = 0$. At equilibrium, $Freq(A_1B_1C_1) \simeq Freq(A_1B_2C_1) \simeq 0.5$. G ranges from 5000 to 150 for $s = 0.1$ to 1.0. There is no disequilibrium between loci A and B. This outcome is the classical case of balanced polymorphism for 2 alleles, each more fit in one habitat. The frequency dependence outlined above also occurs under these conditions.

4. Habitat selection and fitness evolve together. Both loci A and B evolve when $p_A = 0.99$, $s > 0$ and δ, the pre-mating migration coefficient, is equal to zero (i.e. there is complete within habitat mating). This occurs for $\alpha < 0.8$. At equilibrium, $Freq(A_1B_1C_1) \simeq Freq(A_2B_2C_1) \simeq 0.5$. G ranges from 600 to 4500 generations and is positively correlated to s and α. At equilibrium, the population-level disequilibrium is at its maximum (i.e. $D_{tot} \simeq .25$). Almost 100% of D_{tot} is due to allele-frequency differences between the subpopulations in the two habitats (i.e. $D_{bet} \simeq .25$). This outcome represents the evolution of complete habitat specialization, which further results in the splitting of the population into evolutionary

distinct subpopulations. This is essentially the process many authors (e.g. Bush, 1975) propose for host race evolution.

5. Speciation of habitat specialists. All three loci (A, B, and C) evolve if $\alpha = 0.99$, $s > 0$ and $p_A > 0$. At equilibrium, $Freq(A_1B_1C_1) \simeq Freq(A_2B_2C_2) \simeq 0.5$, although at lower values of s and p_A, the equilibrium genotype frequencies can be less then 0.5, as is shown in Figure 4.3a. This outcome may also be obtained if $\alpha = 0.8$ and $s \geq 0.5$, as is demonstrated in Figure 4.3b. G ranges from 200 to 4200 generations and is negatively correlated with s, p_A and α. D_{tot} ranges from 50-100% of its maximum, and D_{bet} from 0.0 to 0.25, varying strongly and negatively with δ. In this case, however, D_{bet} is not very meaningful, since the two genotypes (rather than habitat subpopulations) are reproductively isolated subpopulations. This outcome represents the evolution of complete reproductive isolation between habitat specialists due to genes which specifically affect mating. Locus C evolves here specifically because the lack of complete penetrance of habitat selection ($p_A < 1.0$) guarantees selection for some mechanism of reproductive isolation between habitat specialists.

6. Polymorphic equilibria. These outcomes may be divided into two categories, according to which genotype (in addition to $A_1B_1C_1$) is most common:

(1) The first category corresponds to outcome 5 above, in which all these loci evolve to equilibrium frequencies of 0.5 for each allele. At equilibrium however, the two most common genotypes ($A_1B_1C_1$ and $A_2B_2C_2$) are equal and at frequencies of less than 0.5. An example

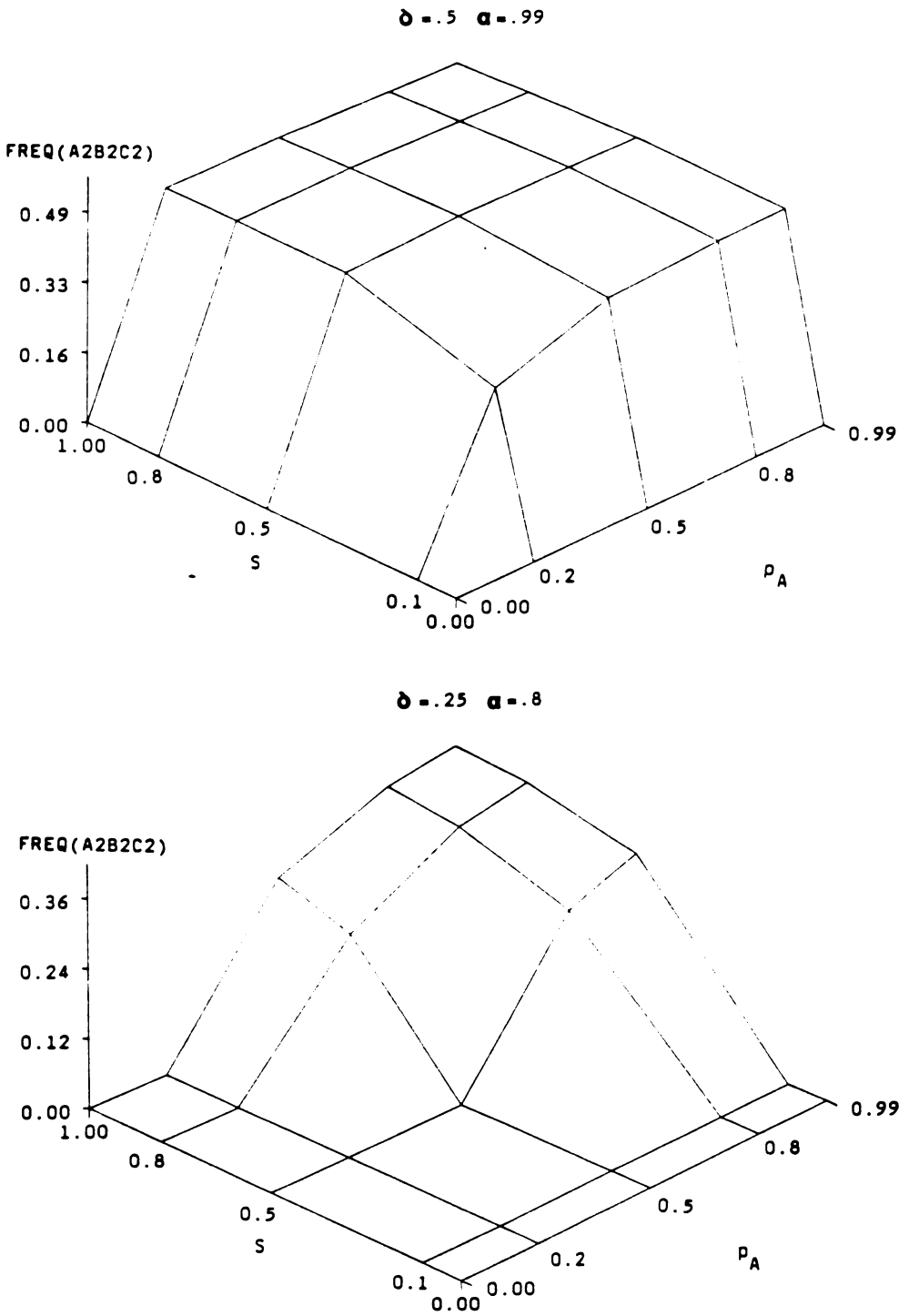


Figure 4.3 One population model simulation. Plots showing the relationships between s, p_A and the equilibrium frequency of the habitat 2-specialist.

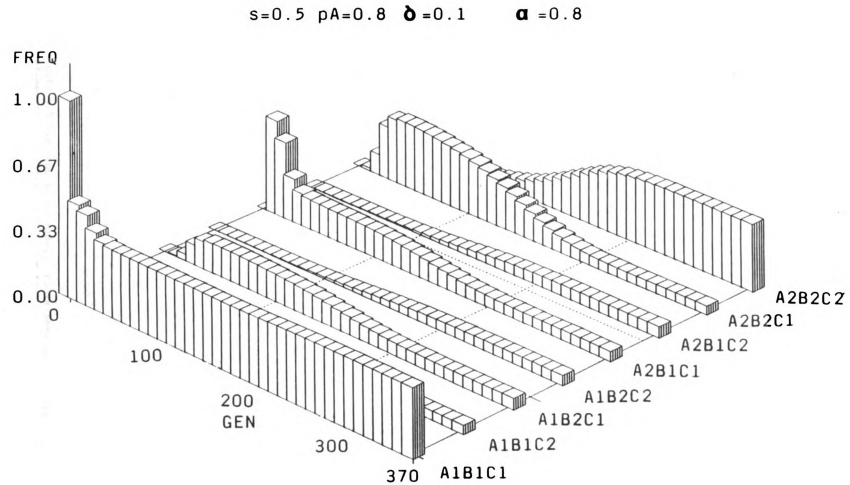


Figure 4.4. Polymorphic equilibrium. Frequencies of all genotypes are plotted every 10 generations for the one population model, with the parameter values set as shown. The frequencies at 370 generations represent equilibrium conditions.

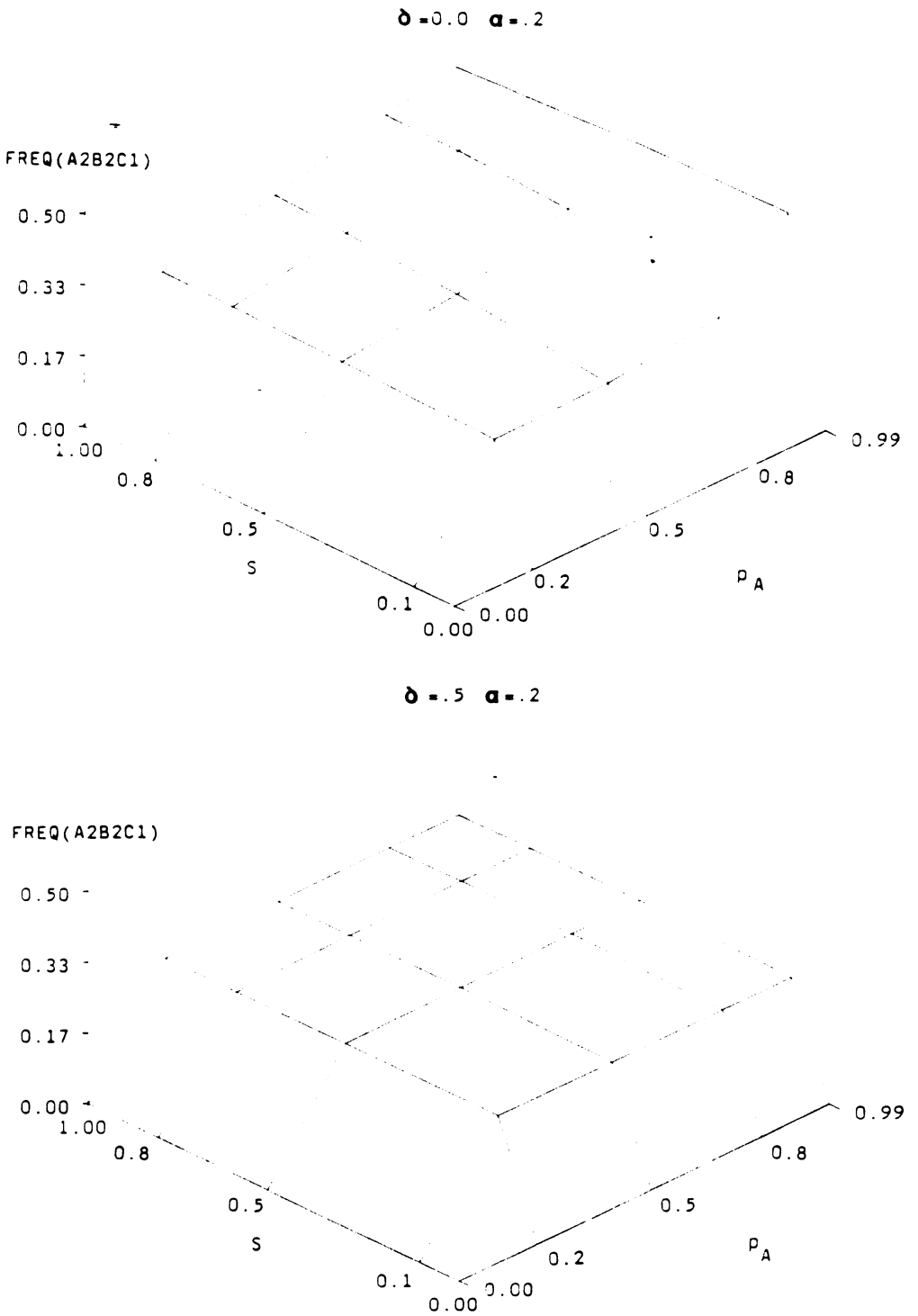


Figure 4.5 One population model. Plots showing the relationships between s, p_A and the equilibrium frequency of the habitat 2 specialist.

of a simulation with this outcome is shown in Figure 4.4, which plots the frequency of all genotypes over time (generations). Figure 4.5 demonstrates the effects that α, s, p_A and δ have on the equilibrium frequency of $A_2B_2C_2$. G varies from 1900 to 200. Once equilibrium has been reached, there is still selection for increased reproductive isolation between specialists. Thus, modifiers which serve to increase the value of α or decrease the value of δ should be favored.

(2) The second category corresponds to outcome 4 above, in which loci A and B evolve to allele frequency equilibria of 0.5, but to four rather than two genotypes. An example of a simulation with this outcome is shown in Figure 4.6, which plots the frequency of all genotypes over time (generations). In general, this outcome is obtained when $\alpha < .8$, $s > 0$ and $p_A > 0$. The equilibrium $Freq(A_1B_1C_1)$ [$\approx Freq(A_2B_2C_1)$] is negatively correlated with δ and p_A, and positively correlated with s. G varies from 4800 to 200. Again, modifiers which serve to increase the value of α or decrease the value of δ should be favored.

In order to obtain a broad view of the effects of the various parameters, the population wide disequilibria, D_{tot}, and the fraction of that contained between populations, $D_{bet\%} = D_{bet}/D_{tot}$, were tested for correlation to the parameters s, p_A, δ, and α by Spearman's rank correlation (Table 4.3). Both D_{tot} and $D_{bet\%}$ were significantly and positively correlated with s and p_A and negatively correlated with δ. α was positively correlated with D_{tot} only.

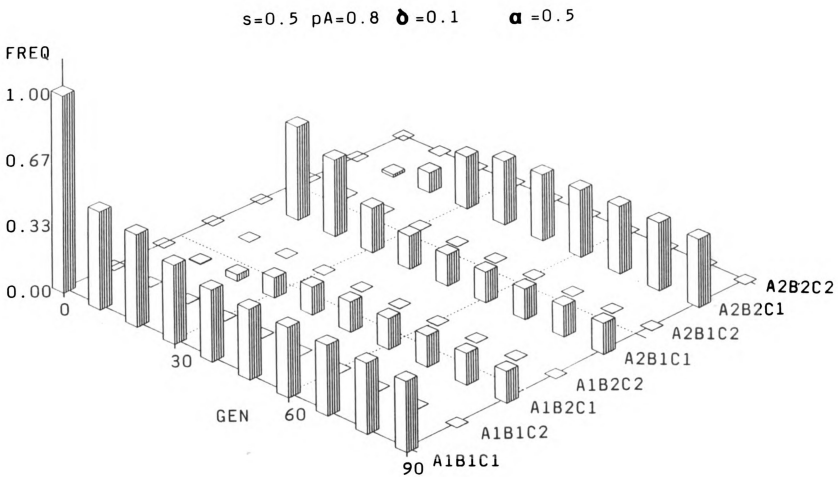


Figure 4.6. Polymorphic equilibrium. Frequencies of all genotypes are plotted every 10 generations for the one population model with the parameter values set as shown. The frequencies at 90 generations represent equilibrium conditions.

Table 4.3. Spearman's correlation coefficients for 4 variables in the one population model and two measures of disequilibrium, D_{tot}, the total disequilibrium between the A and B loci, and D_{bet}/D_{tot}, the proportion of that total due to differences in frequencies between populations. [*** denotes $p < 0.001$, ** denotes $p < 0.01$, and ns denotes $p > 0.05$]

	Measures of Disequilibrium	
Variable	D_{bet}/D_{tot}	D_{tot}
s	0.34 ***	0.55 ***
p_A	0.41 ***	0.57 ***
δ	-0.53 ***	-0.12 **
α	-0.05 ns	0.20 ***

II. Multi-population model

METHODS

In the multi-population model, eleven populations are imagined in a linear array. The dynamics of each population are completely distinct and identical to those outlined in the one population model, with the exception that at the end of each generation, a certain frequency, M, of the genotypes in each population migrate to the adjacent populations. This represents a stepping-stone model of migration between populations. The parameters p_A, s, and δ may vary between populations, while the parameters M and α are constant over all populations. The goal of these simulations was to examine situations in which conditions in the middle set of populations (Pops. 5-8) were appropriate for the evolution of speciation by habitat specialization (outcomes 4 and 5, above), but conditions in the rest of the population did not favor these results (outcomes 1-3). Given the results of the one population simulations, trials were run with the following variations of parameters:

(1) α = 0.0 or .99

(2) M = 0.01 or 0.001

(3) s = 0.5 in all populations OR
s = 0.5 in populations 5-8 and s = 0.0 in all other populations.

(4) p_A = .99 in all populations OR
p_A = .99 in populations 5-8 and p_A = 0.0 in all other populations.

(5) δ = 0.0 in all populations OR
δ = 0.0 in populations 5-8 and δ = 0.5 in all other populations.

Thus s, p_A, and δ can either be constant over all populations or may show a cline (in this case a step function decline) in their values.

When there exits a cline, the parameter values favoring habitat specialization are present in the four middle populations (5-8).

RESULTS

The results fall into 2 categories, depending on whether conditions in the middle populations favor outcomes 4 or 5 above. When $\alpha = 0.99$, speciation rapidly occurs in the middle populations, i.e. genotypes $A_1B_1C_1$ and $A_2B_2C_2$ become fixed at frequencies of 0.5. This condition spreads to the outer populations at a rate equal to the rate of migration between habitats. This occurs because all 2-alleles are either neutral or favored in these outer populations. [The exception to this is when both p_A and s are greater than zero in the outer populations (Figure 4.2a), in which case conditions in the outer populations favor speciation and equilibrium is quickly reached.] The values of δ do not affect the rate of spread. The speciation condition spreads to all populations because the C2-alleles enter these outer populations in the $A_2B_2C_2$ genotype. Since assortative mating is almost perfect, the associations of the 2-alleles is maintained. Since the middle populations constantly send out only the 2 specialist genotypes, all populations eventually reach the $A_1B_1C_1$-$A_2B_2C_2$ equilibrium. How quickly they reach this equilibrium depends directly on the value of M, the rate of migration between adjacent populations. When M = .01, approximately 3500 generation are needed to reach overall equilibrium. A representative simulation after 2000 generations is shown in Table 4.4. The middle populations equilibria are not affected by migration because selection corrects frequencies back to the 50% level for each specialist.

Table 4.4. Genotype frequencies and levels of disequilibrium after 2000 generations for 11 populations in the multi-population model. In this example p_A = 0.99 and ∂ = 0.25 in populations 5-8, while p_A = 0.00 and ∂ = 0.5 elsewhere. s = 0.8 in all populations, M = 0.01 and α = 0.99. D_{tot} is the population-wide level of disequilibrium between loci A and B, and D_{bet}/D_{tot} is the percentage of that disequilibrium due to different allele frequencies between the sub-populations.

	Genotype frequencies									
Pop.	**A1B1C1**	A1B1C2	A1B2C1	A1B2C2	A2B1C1	A2B1C2	A2B2C1	**A2B2C2**	D_{bet}/D_{tot}	D_{tot}
1	**0.18**	0.10	0.15	0.12	0.12	0.10	0.10	**0.12**	0.00	0.002
2	**0.19**	0.09	0.15	0.12	0.12	0.10	0.09	**0.14**	0.02	0.004
3	**0.22**	0.07	0.13	0.12	0.11	0.10	0.07	**0.18**	0.02	0.02
4	**0.29**	0.03	0.10	0.09	0.09	0.09	0.03	**0.27**	0.02	0.06
5	**0.49**	0.00	0.01	0.00	0.00	0.00	0.00	**0.49**	0.24	0.24
6	**0.49**	0.00	0.00	0.00	0.00	0.00	0.00	**0.49**	0.25	0.25
7	**0.49**	0.00	0.00	0.00	0.00	0.00	0.00	**0.49**	0.25	0.25
8	**0.49**	0.00	0.0	0.00	0.00	0.00	0.00	**0.49**	0.24	0.24
9	**0.29**	0.03	0.09	0.09	0.09	0.09	0.03	**0.28**	0.02	0.07
10	**0.21**	0.07	0.12	0.12	0.12	0.11	0.07	**0.20**	0.02	0.02
11	**0.18**	0.08	0.13	0.12	0.12	0.12	0.08	**0.17**	0.01	0.01

Table 4.5. Genotype frequencies and levels of disequilibrium after 2000 generations for 11 populations in the multi-population model. In this example $p_A = 0.99$ and $\delta = 0.00$ in populations 5-8, while $p_A = 0.00$ and $\delta = 0.5$ elsewhere. $s = 0.8$ in all populations, $M = 0.01$ and $\alpha = 0.00$. The frequencies of all C_2-genotypes are all zero.

	Genotype frequencies				Disequilibrium Measures	
Pop.	**A1B1**	A1B2	A2B1	**A2B2**	D_{bet}/D_{tot}	D_{tot}
1	**0.28**	0.28	0.22	**0.22**	--	0.00
2	**0.27**	0.27	0.23	**0.23**	--	0.00
3	**0.27**	0.27	0.23	**0.23**	0.54	0.00005
4	**0.26**	0.25	0.24	**0.24**	0.50	0.0048
5	**0.49**	0.01	0.01	**0.49**	0.99	0.241
6	**0.50**	0.00	0.00	**0.50**	0.99	0.247
7	**0.50**	0.00	0.00	**0.50**	0.99	0.247
8	**0.49**	0.01	0.01	**0.49**	0.99	0.241
9	**0.26**	0.25	0.24	**0.24**	0.50	0.0048
10	**0.27**	0.27	0.23	**0.23**	0.54	0.00005
11	**0.27**	0.27	0.23	**0.23**	--	0.00

When $\alpha = 0.0$, the genotypes $A_1B_1C_1$ and $A_2B_2C_1$ come to equilibrium frequencies of 0.5 in the middle populations. This state is reached in the outer populations only in the trivial cases which occur when $s = .8$, $p_A = .99$ and $\delta = 0.0$ (this is the one-population equilibrium for those conditions). In cases in which either $s = 0.0$ or $p_A = 0.0$, the outer populations reach equilibria at equal frequencies of the four genotypes $A_1B_1C_1$, $A_1B_2C_1$, $A_2B_1C_1$, and $A_2B_2C_1$. The number of generations needed to reach this equilibrium varies directly with M, the rate of migration. At equilibrium, a step function is seen at the cline boundary (i.e. between populations 5 and 4, and 8 and 9) not only in gene frequencies but in the amount of disequilibrium between loci A and B. Table 4.5 demonstrates that the middle populations exhibit almost 100% of the maximum disequilibrium ($\simeq 0.25$), almost all of which can be expressed as between population disequilibrium (D_{bet}). The outer populations exhibit little total disequilibrium, all of which is within populations (i.e. $D_{bet} = 0.0$).

DISCUSSION

The results of the one population simulations show that complete reproductive isolation between habitat specialists can occur only under two sets of conditions: (1) There is a high degree of assortative mating and there are selective differences between habitats. At lower levels of assortative mating disruptive selection must be strong to result in speciation. (2) There must be high penetrance of habitat selection and a high degree of mating within habitats. This results in high between-subpopulation levels of disequilibrium between the selection and habitat selection loci. These two major results of this model are congruent with the results of

models of Felsenstein (1981) and Bush and Diehl (1989), respectively. While this model does not include certain variations of these models (i.e. multiplicative fitness functions and variable linkage between loci), it is a general model exploring the parameter areas between the extremes which these models represent. Furthermore, it provides a basis for building a model to explore the effects of migration on the evolution of habitat specialization under a large number of parameter combinations.

The results of the multi-population models confirm that there are conditions under which newly evolved habitat specialists will spread slowly (or will fail to spread) to areas where selective and population structure conditions are insufficient. Whether of not specialists spread depends on the method of reproductive isolation evolved between habitat specialists.

If speciation has evolved based on true assortative mating (rather than simply strict habitat use and mating in the habitat), newly evolved habitat-specialist species will spread only at migration frequency to areas in which either the selective differential between habitats or the penetrance of habitat selection falls to zero. For example, if penetrance falls off at some cline, despite differential fitness of the alternative A-locus alleles in the habitats, the new specialist species does not increase in frequency. Because of the lack of penetrance of habitat selection, it ends up in its 'bad' habitat as often as in its 'good' one. It cannot increase in frequency due to its higher adaptation to one habitat's conditions. The only increase in frequency of this newly evolved species is caused

by migration. However, since the middle population constantly sends out individuals of this species, the species will become fixed, given time, in all populations. The rate of fixation depends directly on the rate of migration between populations. This outcome should also apply to species which have diverged allopatrically, or even unrelated species which specialize on certain habitats.

How does this result affect my interpretation of the P. carabi-Nicrophorus pattern seen at my two study sites? A limited number of laboratory crosses suggest strongly that the specialist mites at KBS do not successfully produce hybrid offspring. Whether this indicates assortative mating by these mites or a post-mating barrier (such as inviability of offspring) is not known, but let us assume the former for the moment. There is a clear drop in the penetrance of habitat selection as one moves north from KBS, due to the competitive interactions of the beetles, N. orbicollis and N. defodiens. Mites riding on N. defodiens to a carcass are not assured of reproducing with N. defodiens, since these carcasses are often taken over by N. orbicollis. While there is a drop in the penetrance of habitat selection at the N. defodiens boundary, the real conditions do not conform exactly to the models conditions. The change in penetrance of habitat selection is unidirectional, i.e. mites riding on N. defodiens are often shifted to an N. orbicollis environment but not vice versa. Thus for mites on N. defodiens the degree of mating in the habitat (δ) and, more importantly, the penetrance of habitat selection (p_A) is altered, but for mites on N. orbicollis only δ is changed. δ should not affect the spread of the orbicollis-specialist when assortative mating is strong. The orbicollis-specialist should

find itself in the appropriate environment (i.e. with N. orbicollis) and thus increase in frequency above the basal level of migration. Unless migration or the time since sympatric divergence is extremely low, the model incorporating strict assortative mating would not appear to explain the lack of the orbicollis-specialist at UMBS.

The conclusions of this model would be significantly altered how if a different (and perhaps more realistic) model of assortative mating were used, for example if assortative mating has a cost when the mating type is in low frequency. Low frequencies of the alternate mating type are expected in the outer populations since the decrease in penetrance of habitat selection (p_A) or disruptive selection (s) means that mating types are mixed between habitats. This cost could compensate for the constant input of habitat specialists from the middle populations and prevent or slow the spread of the new species. Another way to alter assortative mating is for the degree assortative mating (α) to change with the frequency of the mating type. This flexibility of assortative mating would break up the associations of appropriate loci (i.e. the 2-alleles) at the clinal border. In general, models incorporating real individuals instead of population frequencies could radically change the predictions concerning the spread of mating types, because of the effect of drift on genotypes at low frequencies.

Finally, let us consider the situation under which mating is not strictly assortative, but that speciation has evolved based on very strict habitat use. The isolation of the two subpopulations using different hosts has resulted in the production of inviable hybrids

between the two habitat specialists. The degree of isolation between populations dissolves, however, at the clinal boundary (as is shown by the rapid drop in the level of disequilibrium between loci A and B (D_{tot} and D_{bet}, Table 4.5). This scenario is consistent with the KBS-UMBS pattern, and in addition makes some predictions about what morphological patterns one would expect to see across the boundary where N. defodiens is present. If we assume that morphological differences between the specialists are due to isolation, these differences should break down at the N. defodiens-border. Specific assumptions about the genetic control over morphology and the selective value of morphological differences would determine what sort of within-species morphological variation would be found north of the N. defodiens-border.

The strength of this model lies in its potential to cover a range of possible ecological situations. Examples include simulations incorporating habitats of different size, competition between a generalist and a specialist genotype, invasion of a community by a specialist of a different versus the same species, and evolution of specialization in 'pockets of selection' of varying size. The basic model presented here will allow me to explore these permutations, and relate the results to non-genetic models of ecological specialization (e.g. Rosenzweig 1979, Pimm and Rosenzweig, 1981).

CHAPTER 5. PRELIMINARY STUDIES AND RECOMMENDATIONS

In the Chapter 3, two evolutionary hypotheses were proposed to explain the shift in beetle species use by Poecilochirus carabi over the focal study sites in Michigan, the Kellogg Biological Station (KBS) and the University of Michigan Biological Station (UMBS). Both of these scenarios invoke the presence of the beetle species Nicrophorus defodiens at UMBS as the crucial element in preventing the existence of the orbicollis-specialist at that site. In the "patchy divergence" scenario, the presence of N. defodiens disrupts the conditions of selection and population structure necessary for evolutionary divergence between habitat specialists to proceed. In the local competition scenario, the presence of N. defodiens results in a shifting of mites from N. defodiens and N. tomentosus to N. orbicollis. The presence of a large number of mites shifted onto N. orbicollis depresses overall fitness on that beetle species below the necessary level for population maintenance of a specialist using only N. orbicollis. One would predict that, under either scenario, the distribution of the orbicollis-specialist to correlate negatively with the presence of N. defodiens in the beetle community. Alternatively, if abiotic factors, e.g. temperature, limit the northern distribution of this mite, one should not expect the northern limit of the orbicollis-specialist to coincide necessarily to the southern limit of N. defodiens in Michigan. In order to test this prediction, I have begun a preliminary survey of the morphology of mites caught on N. orbicollis in areas in lower Michigan between KBS and UMBS.

In this chapter I also present the results of preliminary studies

of the behavior and morphology of mites at sites outside Michigan. The goals of these studies were to compare the degree of behavioral and morphological variation seen in P. carabi in Michigan to mites from sites which support different sets of beetle species in the carrion community. I consider the range of morphological variation in size and setal lengths for mites from a number of western U.S. and S. American Nicrophorus species. I also present detailed results of behavioral trials and morphological measurements from southwestern South Dakota. The results support my contention that P. carabi is variable in its host use. They also provide insight into the types of data needed to corroborate the evolutionary scenarios I have proposed to explain this local variation in specialization.

I consider these studies preliminary because they involve, for the most part, a collection of mites and beetles from a site on a single sampling date. As my studies at KBS and UMBS have demonstrated, mite preferences for beetle species may shift over the season, and thus a single set of behavioral trials may not represent the true host use strategy of mites from one location. Finally, the interpretation of any results is contingent on a thorough understanding of the ecology of the local beetle community, which is lacking in the case of the Missouri and South Dakota sites. Thus, conclusions from this work should be considered as preliminary and a guide for future investigations.

MATERIALS AND METHODS

Michigan transect -- Two transects between KBS and UMBS were run on separate dates using the standard trapping procedures (Chapter 2).

On July 7, 1988, beetles were trapped at seven sites which spanned the distance between KBS and UMBS (sites T1 through T7 in Figure 5.1). Three traps were placed at least 0.3 miles apart at each site (Table 5.1). After determining that this was the likely area containing the southern limit of N. defodiens, a second transect spanning the distance between T2 through T4 was run on August 8, 1988 (Table 5.2). On both dates traps were set moving from south to north (e.g. T1-T7) during the afternoon, and beetles were collected during the following morning and afternoon. In the case of the KBS-UMBS transect, the traps were collected in reverse order (T7-T1). Since the focal beetles species, N. defodiens and N. orbicollis, fly during the hours 1800-2400 (Wilson et al. 1984), this difference in the amount of time the traps were set should not effect the number of beetles of these species caught, although it should affect the numbers of N. tomentosus caught. Nicrophorus spp. were collected from the traps and placed in 4.5 liter plastic shoe boxes (maximum 25 beetles/box) with moist towelling and held for 24 hours at 19^{o} C. for 24 hours. Mite densities were determined by counting under a magnifying glass (densities greater than 10 were verified by counting under a dissecting microscope after beetles and mites were preserved). Beetle species preference tests were performed where enough beetles were captured and analyzed using the methods described in Chapter 2.

Morphological analysis was performed on the mites found on N. orbicollis across the transect. As was done with KBS and UMBS mites, mites were submitted to two morphological measures, body size and podonotal setal lengths. Body size was determined by measuring the

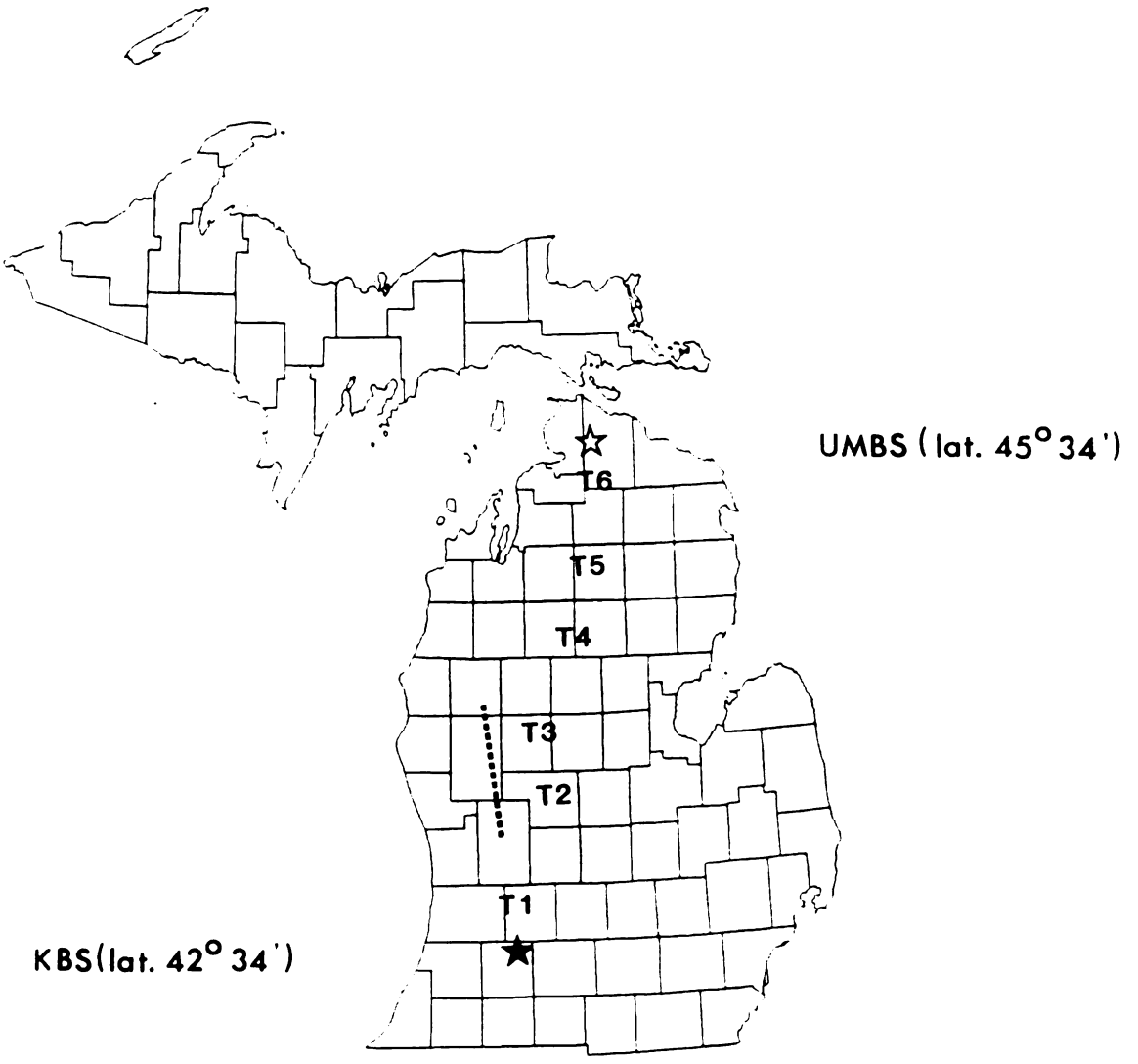


Figure 5.1. Map of trapping sites for transects between KBS and UMBS. The latter two sites are indicated by stars. The approximate sites for the second transect in August are shown by the dotted line.

Table 5.1. Locations of trapping sites for transect between KBS and UMBS on July 7, 1988.

MICHIGAN TRANSECTS

Site	Location	Latitude
T6	1 km SE of Indian River, Cheboygan Co.	45° 24′
T5	4 km NE of Frederick, Crawford Co.	44° 48′
T4	7 km NW of Houghton Lake, Roscommon Co.	44° 20′
T3	7 km SW of Barryton, Mecosta Co.	43° 42′
T2	4 km S of Stanton, Montcalm Co.	43° 15′
T1	2 km SW of Yankee Springs, Barry Co.	42° 36′

Table 5.2. Locations of trapping sites for transect between sites T2 and T3 (see Table 5.1a) on August 8, 1988.

M1	6 km NE of Nirvana, Lake Co.	43o 57′
M2	3 km NE of Nirvana, Lake Co.	43o 55′
M3	4 km SW of Nirvana, Lake Co.	43o 53′
M4	9 km SW of Nirvana, Lake Co.	43o 50′
M5	4 km W of Hawkins, Newaygo Co.	43o 48′
M6	3 km W of Parks, Newaygo Co.	43o 47′
M7	6 km W of Big Rapids, Mecosta Co.	43o 41′
M8	6 km W of Stanwood, Mecosta Co.	43o 34′
M9	9 km SW of Stanwood, Mecosta Co.	43o 32′
M10	9 km W of Morley, Mecosta Co.	43o 29′
M11	4 km E of Croton Heights, Newaygo Co.	43o 27′
M12	1.5 km SE of Croton, Newaygo Co.	43o 26′
M13	7 km ESE of Newaygo, Newaygo Co.	43o 24′
M14	9 km SE of Newaygo, Newaygo Co.	43o 22′
M15	8 km NNE of Kent City, Kent Co.	43o 17′
M16	6 km Ne of Kent City, Kent Co.	43o 16′
M17	6 km E of Kent City, Kent Co.	43o 14′
M18	5 km NNE of Sparta, Kent Co.	43o 13′

length and width of the sternal shield, which shows the same size relationships between morphs as the dorsal shields. [An analysis of variance on sternal shield size of mites from the reproductive success experiments demonstrated that while orbicollis-specialists were generally larger than tomentosus-specialists, body size was affected in both morphs by the associated beetle species.] In Chapter 2, the differences between KBS orbicollis- and tomentosus-specialists in setal lengths were obtained by measurements of 19 podonotal setal lengths, which were then used as variables in a principal components analysis (PCA). Because of the smaller number of mites available from the transect studies, and the propensity for setae to fall off during preparation and mounting of the mites, it was necessary to use fewer setal measurements in this morphological analysis to ensure a large enough sample size. A subset of the 19 setae was chosen which highly characterize the differences between the specialist morphotypes. Table 2.5 in Chapter 2 summarizes the distributions of setal lengths among the mites found on KBS N. tomentosus and N. orbicollis. I chose to measure 4 setae, i3, i5, z2, and z4, which demonstrated the least degree of overlap in range between the two morphs. When these four setae alone are used in a PCA, enough mites from the transects can be measured. However, some degree of accuracy is lost in the ability to discriminate between mites of the two morphs. This is shown in Figure 5.2, which plots that results of a PCA using the lengths of the four setae above. The data set is the same as in Figure 2.6, which plotted 19-setae PCA of mites from KBS and UMBS. In Figure 2.6, the KBS specialists fell into distinct clouds and the UMBS mites fell clearly into the tomentosus-specialist cloud. In Figure 5.2 most of the UMBS mites fall into the tomentosus-specialist cloud, but many are

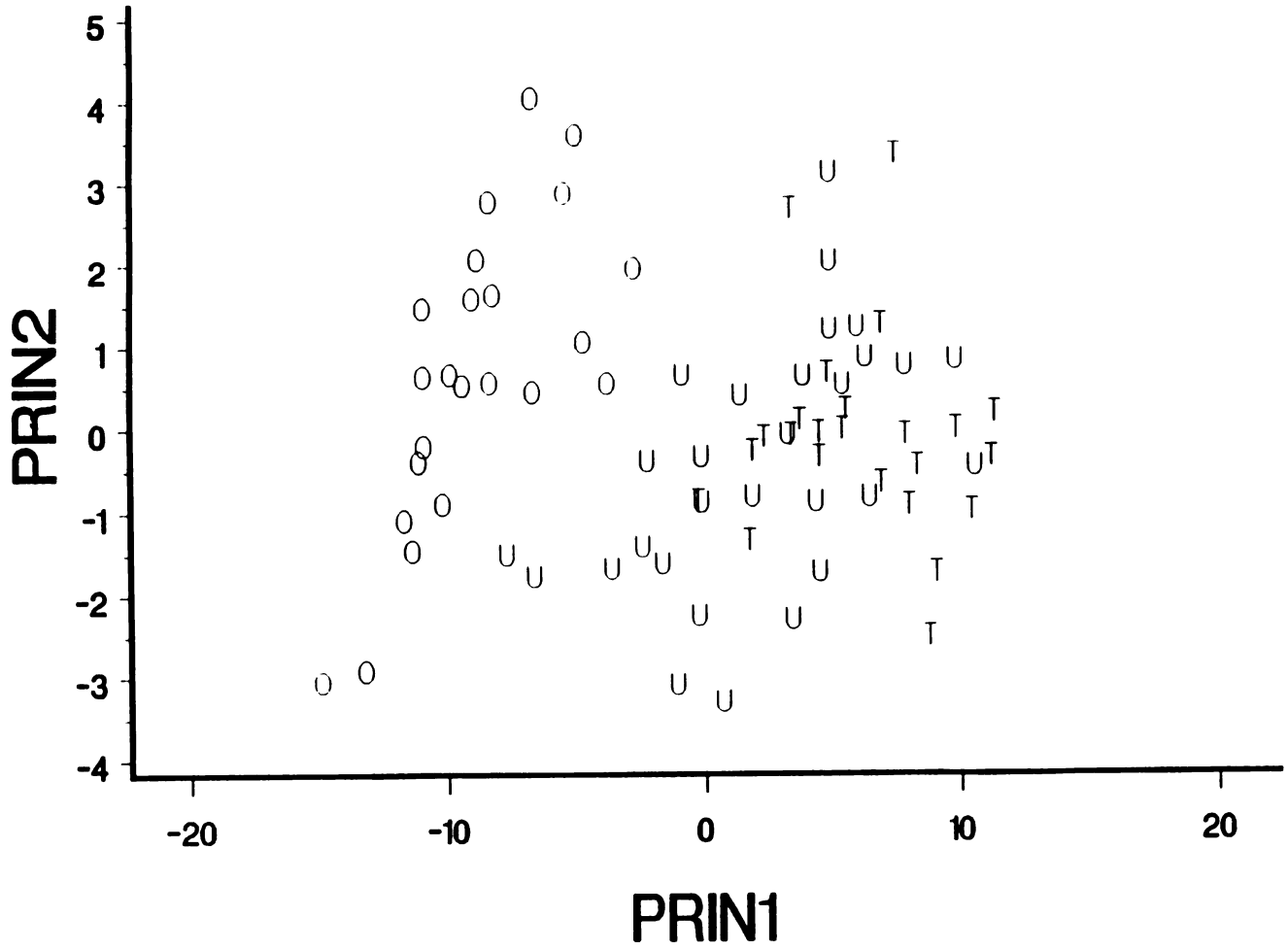


Figure 5.2. Principle components analysis on setal lengths of mites from reproductive success experiments (Chapter 3). This analysis used 4 setae (i3 , i5, z2 and z4). The first and second principle components (PC1 and PC2) explained 92% and 4% of the variance, respectively. O, T and U refer to orbicollis-specialists, tomentosus-specialists, and UMBS mites (from several species), respectively.

also intermediate. In other words, the necessity of using fewer setae in this analysis results in some loss of information. This should be taken into account when considering the results.

Non-Michigan sites -- The locations of the site are given in Table 5.4. Collection techniques were identical to those described in Chapter 2. The exceptions to this are the mites from Chile and Peru, which were obtained from trap residues stored at the Field Museum of Natural History in Chicago. In these cases, it was known that Nicrophorus beetles were taken in the same traps, but since P. carabi fall off beetles when preserved and many insect species are found in these traps, the association of these mites with Nicrophorus species is not absolutely certain. Behavioral (beetle preference) tests were performed where possible according to the methods given in Chapter 2. Lengths and widths of the sternal shield and lengths of the dorsal podonotal setae were measured using an ocular micrometer at 200x. Analysis of differences in body size and setal morphometry were performed as above, except that a larger subset of podonotal setae (i3, i5, z2, z3, z4, s3, and s4) were able to be used in the principal components analysis of setal size patterns.

RESULTS

Michigan transect -- The numbers of beetles of each species and the associated densities of P. carabi are given in Table 5.3 for each site in the two trapping transects. Because of improper care, mite densities from T1 could not be used in this analysis. The numbers of beetles caught at each site varied greatly, presumably reflecting the suitability of the local habitat. There were no obvious trends in

Table 5.3 -- P. carabi densities. Number of beetles (n), mean number of mites/beetle + 1 standard deviation (Density) of mites on three beetle species caught at the indicated Michigan transect sites in 1988. Site M is an average of all sites in the second transect (M1-M18).

	N. orbicollis		N. tomentosus		N. defodiens	
Site	n	Density	n	Density	n	Density
T6	33	2.9 + 2.6	10	28.5 + 14	3	34.0 + 6.1
T5	43	1.4 + 2.3	16	19.1 + 8.8	7	9.0 + 7.0
T4	1	0.0	0	----------	2	17.0 + 1.4
T3	7	7.0 + 4.8	3	13.7 + 8.1	4	5.3 + 2.4
M	41	3.2 + 3.8	50	6.7 + 6.9	11	6.6 + 9.4
T2	1	26.0	4	0.25 + 0.5	0	----------

mite densities, except that aside from site T3, N. defodiens and N. tomentosus carried higher densities of mites than N. orbicollis. N. defodiens were caught as far south as site T3 (lat. 43° 42′) in the first transect. In the second transect, 9 N. defodiens were caught at site M4 (lat. 43° 50′) and a single N. defodiens individual at site M18 (lat. 43° 13′). At this longitude, then, the southern limit of N. defodiens appears to lie between sites T2 and T3, i.e. just above the 43rd parallel in latitude.

Beetle preference tests were run on mites from sites T2, T5 and T6 (Figure 5.3). While the number of trials are often too small for statistical significance, the trend across sites is what might be expected given the earlier results at KBS and UMBS. Mites from N. orbicollis at site T2 behave as KBS mites do, highly preferring N. orbicollis over N. tomentosus. Mites from N. orbicollis at sites T5 and T6 apparently prefer N. tomentosus or N. defodiens to N. orbicollis, as was shown for UMBS mites (Figure 2.2).

The results of the sternal shield size analysis of mites found on N. orbicollis demonstrate a shift from sizes typical of orbicollis-specialists to those typical of tomentosus-specialists as one moves north from KBS to UMBS. The sizes of the KBS specialists and UMBS mites from the reproductive success experiments are shown for reference alongside the transect mites Figure 5.4. The greatest variation in size occurs at sites 2.5 (i.e. the transect between sites T2 and T4) and T3. As stated above, this the approximate southern limit of N. defodiens.

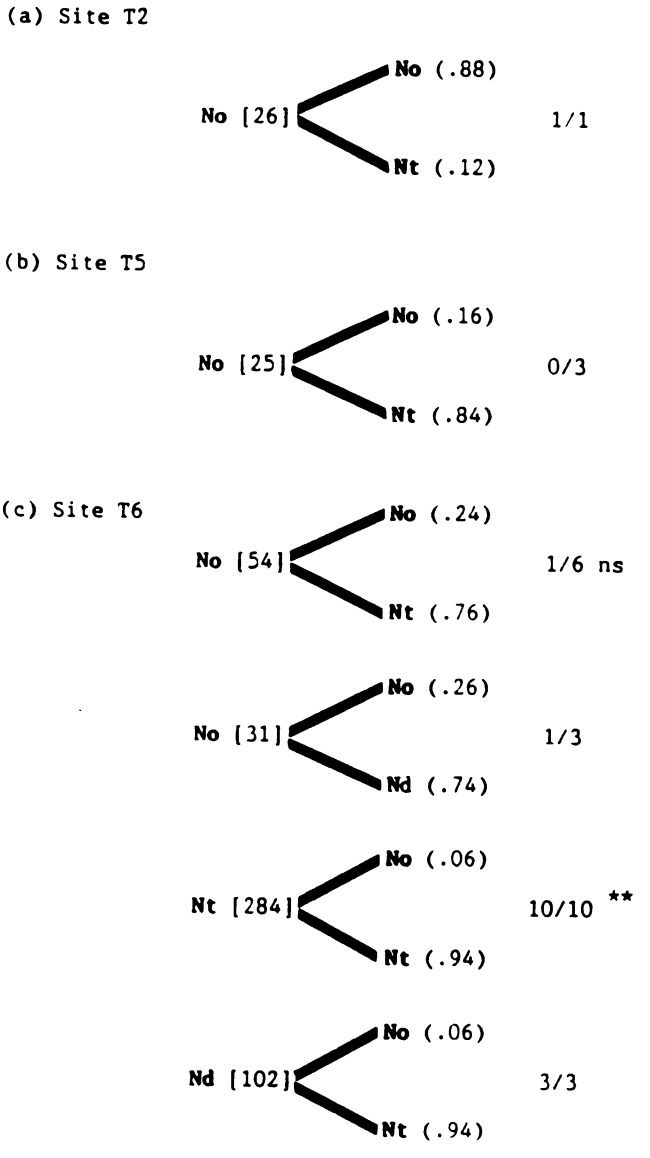


Figure 5.3 Pairwise behavioral preferences tests on mites and beetles from transect sites T2, T5 and T6. No, Nt, and Nd refer to N. orbicollis, N. tomentosus and N. defodiens, respectively. Mites from the species at the node of the branch were offered a choice between the species at the ends of the branches. The total number of mites tested is shown in brackets and the percent of these which chose each beetle species in indicated in parentheses. In each trial, a 'win' by one species occured if that species had a higher number of mites than the alternative. The fraction of trials 'won' by the species from which mites were derived is shown at the right of each branch. Statistical significance was tested by Chi-square analysis when enough trials in a set were performed that a statistically significant result could have been obtained (i.e. # of trials > 4). [** $p < 0.01$]

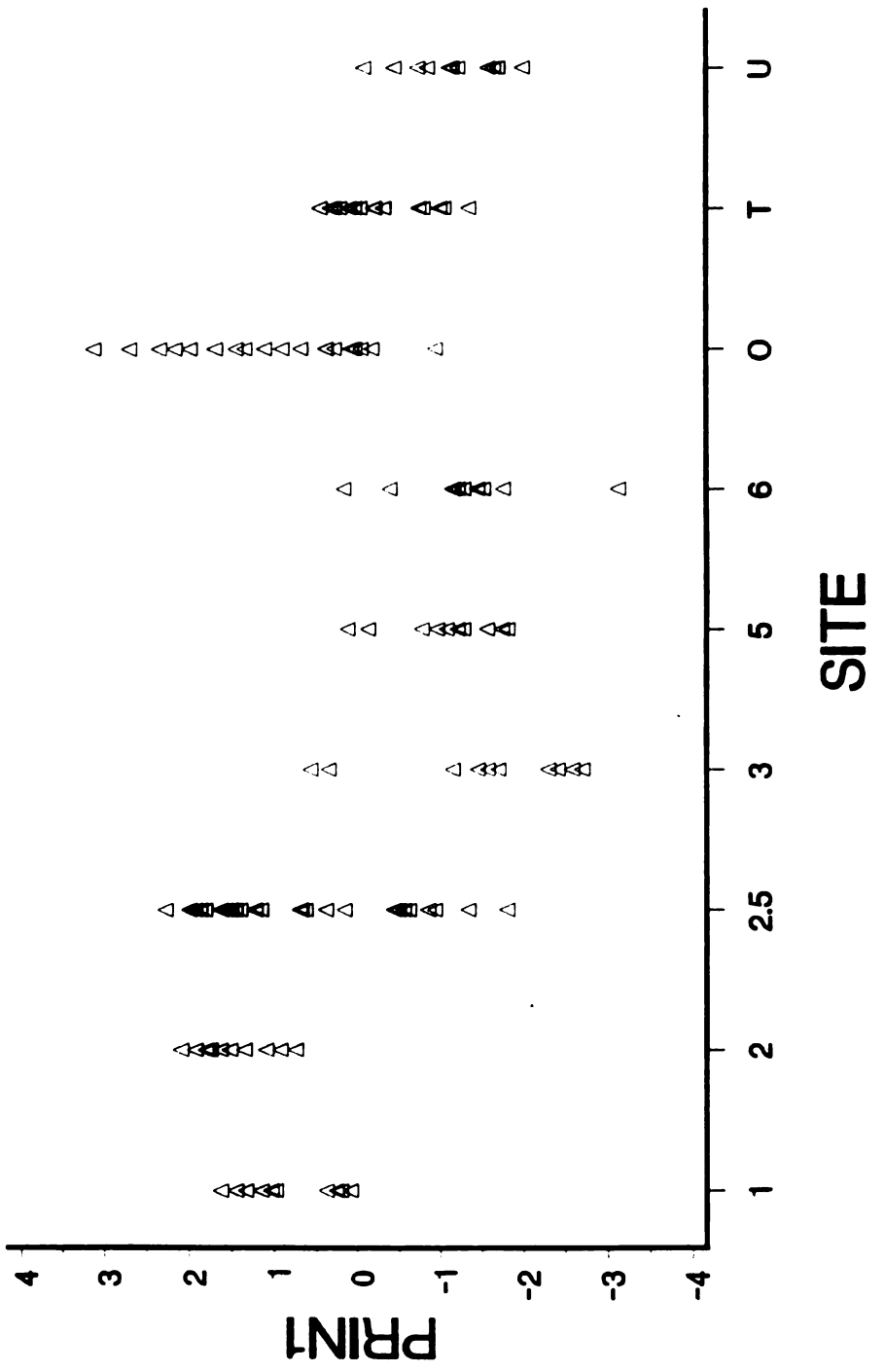


Figure 5.4. Principal components analysis of body size based on the length and width of the sternal shield. The first principle componant, which accounts for 89% of the variance, is plotted for all individuals measured at all transect sites (1 = T1, 2 = T2, etc.). Site 2.5 represents mites captured from the second transect (i.e. M1-M18). O, T and U refer to orbicollis-specialists, tomentosus-specialists and UMBS mites (from several species), respectively, all raised in the laboratory in the reproductive success experiments (Chapter 3).

The results of the 4-setae PCA on transect mites is shown in Figure 5.5, which plots the first principal component across all sites. The PC scores of the KBS specialists and UMBS mites from the reproductive success experiments (Chapter 3) are plotted alongside for comparison. Unlike the PCA for sternal size, the first principal component in this analysis clearly distinguishes the two KBS specialists. The latitudinal pattern is also clear. Mites from sites T1 and T2, south of the range of N. defodiens clearly fall into the orbicollis-specialist category. Mites from the southernmost sites where N. defodiens is captured, sites 2.5 and T3, fall into both morphological categories. Mites from sites T5 and T6 fall into the tomentosus-specialist category, with one exception. A shift in setal lengths from the orbicollis-specialist to tomentosus-specialist morphotype occurs roughly where N. defodiens enters the beetle community.

Non-Michigan sites -- Densities of P. carabi on beetles captured in areas west of Michigan during 1988 are shown in Table 5.4. Tests for mite preference for beetle species were run at those sites at which multiple beetle species were captured, i.e. McGaffey, NM, Bend, OR and Farmingdale and Hill City, S.D. While there were too few tests made to allow confident interpretation of the results, there were indications of host preference for certain beetle species. In New Mexico, N. guttula was more attractive than N. mexicanus. In Oregon, N. nigrita and N. defodiens were more attractive than N. investigator and N. guttula.

A full set of behavioral tests were performed on mites and beetles

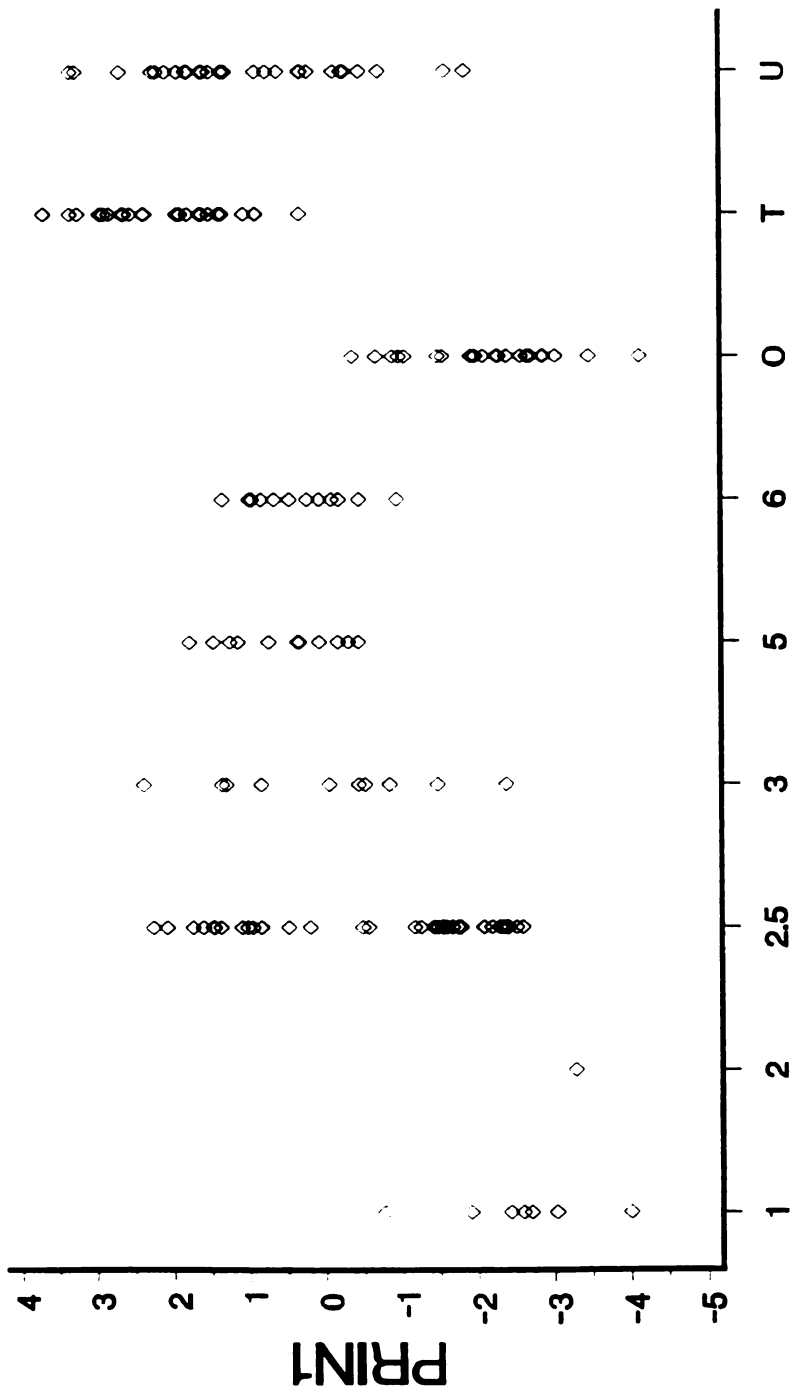


Figure 5.5. Principal components analysis of setal morphometry based on the lengths of setae i3, i5, z2 and z4. The first principle componant, which accounts for 87% of the variance, is plotted for all individuals measured at all transect sites (1 = T1, 2 = T2, etc.). Site 2.5 represents mites captured from the second transect (i.e. M1-M18). O, T and U refer to orbicollis-specialists, tomentosus-specialists and UMBS mites (from several species), respectively, all raised in the laboratory in the reproductive success experiments (Chapter 3).

Table 5.4. Densities of *P. carabi* on beetle species caught during May through July, 1988 at sites outside Michigan. Shown is the site, beetle species, number of beetles caught, and the mean density of mites ± one standard error.

Site	Beetle species	n	Mean Density of *P. carabi*
Mill Creek, MO	*N. orbicollis*	15	4.3 ± 0.9
McGaffey, NM	*N. mexicanus*	3	5.0 ± 1.5
	N. guttula	7	5.3 ± 2.7
Cascade Head, OR	*N. defodiens*	57	3.9 ± 0.5
Blue River, OR	*N. defodiens*	34	6.5 ± 1.2
Bend, OR	*N. investigator*	1	1.0
	N. nigrita	1	10.0
	N. guttula	3	0.0
Hill City, SD	*N. defodiens*	34	10.0 ± 1.0
	N. tomentosus	17	22.6 ± 2.2
Farmingdale, SD	*N. orbicollis*	27	3.4 ± 0.5
	N. tomentosus	34	5.0 ± 0.9

from both South Dakota sites (Figure 5.6). At the Hill City site, in the Black Hills, I captured N. defodiens and N. tomentosus, while at the lower elevation Farmingdale site N. orbicollis, N. marginatus and N. tomentosus were captured (Table 5.4). Mites from N. marginatus strongly prefer this beetle species over all others. Morphological examination indicated that these mites are highly differentiated from P. carabi and most closely resemble the species P. longisetosa. This species is described in an unpublished thesis by Yoder (1972), describes them as occurring on N. marginatus and N. tomentosus from Western Michigan. I have found this mite only on N. marginatus in Michigan (pers. obs.). Mites from the Hill City site highly prefer N. tomentosus over N. defodiens, despite the fact that these two beetle species carry relatively equal numbers of mites on average.

N. tomentosus and N. orbicollis carry moderate numbers of mites at the Farmingdale, SD site (Table 5.4). Preference tests indicate that mites from N. tomentosus prefer this species 3 to 1 over N. orbicollis, but mites from N. orbicollis have no significant preference for either species (Figure 5.6). Morphological analysis of body size indicated no significant differences between mites taken from or choosing either of these two beetle species in the preference experiments (Table 5.5). Analysis of setal morphometry indicated no significant differences for the first principle component, but the analysis of variance on the second principle component indicated significant main and interaction effects (Table 5.6). This result is better interpreted by examining a plot of the second principle component scores from mites in each of the behavioral categories (Figure 5.7), which indicates that mites which were taken from N.

(a) Hill City, SD

(b) Farmingdale, SD

Figure 5.6. Beetle species preference tests on beetles and mites from (a) Hill City, SD and (b) Farmingdale, SD. See Figure 5.3 for explanation of the symbols.

Table 5.5. Analysis of body size (sternal shield size) of mites from Farmingdale, SD preference tests (N. orbicollis vs. N.tomentosus). (a) Eigenvectors generated by principle components analysis. (b) Analysis of variance on principle components scores. Effects represent the beetle species on which mite were found (FROM), and the beetle species they chose in preference tests (CHOSE).

(a) Principle components analysis

	Eigenvectors	
Variable	Prin1	Prin2
Length of sternal shield	0.92	0.39
Width of sternal shield	0.92	-0.39

Proportion of variance -- Prin1 = 0.85

Prin2 = 0.15

(b) Analysis of variance

Prin1

Source	d.f.	SS	F
FROM	1	9.3	0.94 ns
CHOSE	1	5.0	0.50 ns
FROM X CHOSE	1	7.8	0.80 ns
Error	69	679.5	

Prin2

Source	d.f.	SS	F
FROM	1	3.1	1.77 ns
CHOSE	1	2.2	1.25 ns
FROM X CHOSE	1	0.2	0.12 ns
Error	69	119.0	

Table 5.6. Analysis of dorsal setal lengths of mites from Farmingdale, SD preference tests (N. orbicollis vs. N.tomentosus). (a) Eigenvectors generated by principle components analysis. (b) Analysis of variance on principle components scores. Effects represent the beetle species on which mite were found (FROM), and the beetle species they chose in preference tests (CHOSE). [* p < 0.5, ** p < 0.01]

(a) Principle components analysis

	Eigenvectors	
Variable	Prin1	Prin2
i3	0.37	0.02
i5	0.26	0.47
i6	0.23	0.52
z2	0.43	-0.17
z3	0.25	-0.63
z4	0.42	0.20
s3	0.42	-0.18
s4	0.38	-0.09

Proportion of variance -- Prin1 = 0.68

Prin2 = 0.12

(b) Analysis of variance

Prin1

Source	d.f.	SS	F
FROM	1	7.4	0.36 ns
CHOSE	1	0.00	0.00 ns
FROM X CHOSE	1	1.1	0.82 ns
Error	31	572.0	

Prin2

Source	d.f.	SS	F
FROM	1	23.0	6.83 *
CHOSE	1	16.0	9.06 **
FROM X CHOSE	1	21.5	12.2 **
Error	31	49.0	

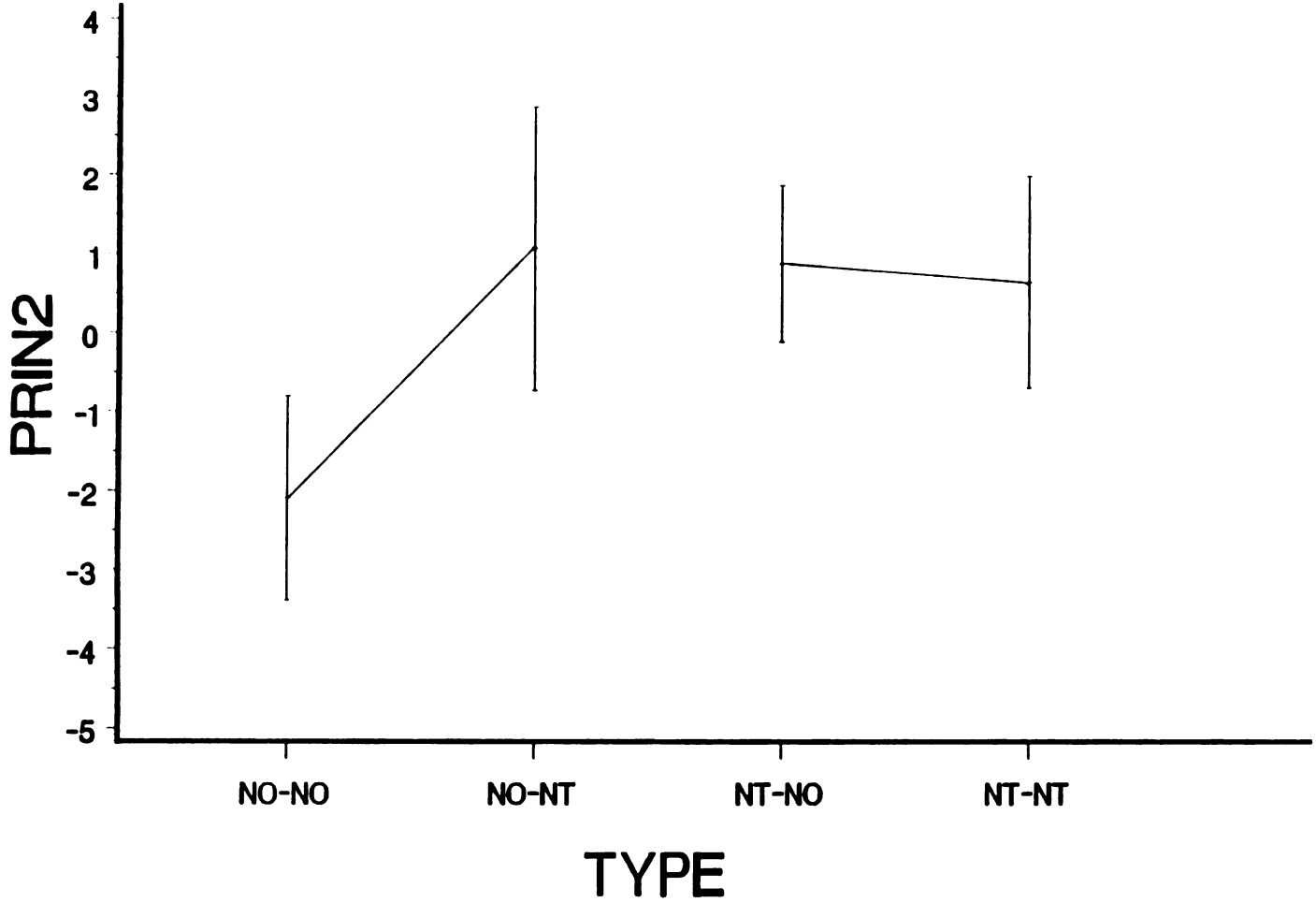


Figure 5.7 Principle components analysis of 8 setal lengths (i3, i5, i6, z2, z3, z4, s3 and s4) from mites from the Farmingdale, SD behavioral preferences tests. The second principle component (mean +- one standard deviation is plotted against the behavioral type, defined by the beetle species on which the mite was found (FROM) and the beetle species chosen in the preference trial (CHOSE). No and Nt refer to N. orbicollis and N. tomentosus, respectively.

orbicollis and then chose N. orbicollis in the preference trials have much lower scores than all other mite types. While there does appear to be some differentiation between South Dakota mites, this differentiation DOES NOT represent the same kind of variation seen between KBS specialists (see Figure 5.9). The KBS orbicollis-specialist morphology is not found on South Dakota N. orbicollis (see Figures 5.8 and 5.9). These results stand in sharp contrast with the KBS preference experiments (Figure 2.4) and morphological analysis (Figure 2.6). The two sites are apparently identical in Nicrophorus species composition, but mites at Farmingdale, SD do not appear to be of two highly distinct behavioral or morphological types, as they do at KBS.

In order to obtain a large picture of morphological diversity in P. carabi, I performed a PCA on body (i.e. sternal shield) size and setal lengths for all P. carabi I have obtained. The eigenvectors for these analyses are shown in Table 5.7. The wide geographical variation in body sizes is apparent (Figure 5.8). The largest body sizes are found in Michigan mites from N. orbicollis. It is apparent from this graph that mites from Missouri fall into two groups; my observation of their general morphology indicates that these two groups closely resemble the orbicollis- and tomentosus-specialists found at KBS. Mites from N. guttula, N. defodiens (Oregon) and N. didymus (Peru) are intermediate between the sizes of the KBS specialists. The analysis of setal lengths provides a clearer picture of geographical differences and similarities (Figure 5.9). In this analysis, the KBS specialists overlap slightly in their ranges of values. Notable are the large values for mites from N. guttula, N.

Table 5.7. Analysis of dorsal setal lengths of mites from all field sites. Eigenvectors of the first two principal are shown for the lengths of 8 setae.

	Eigenvectors	
Variable	Prin1	Prin2
i3	0.40	-0.21
i5	0.37	-0.46
i6	0.31	-0.35
z2	0.35	0.10
z3	0.26	0.41
z4	0.38	-0.13
s3	0.35	0.64
s4	0.40	0.12

Proportion of variance -- Prin1 = 0.89

Prin2 = 0.04

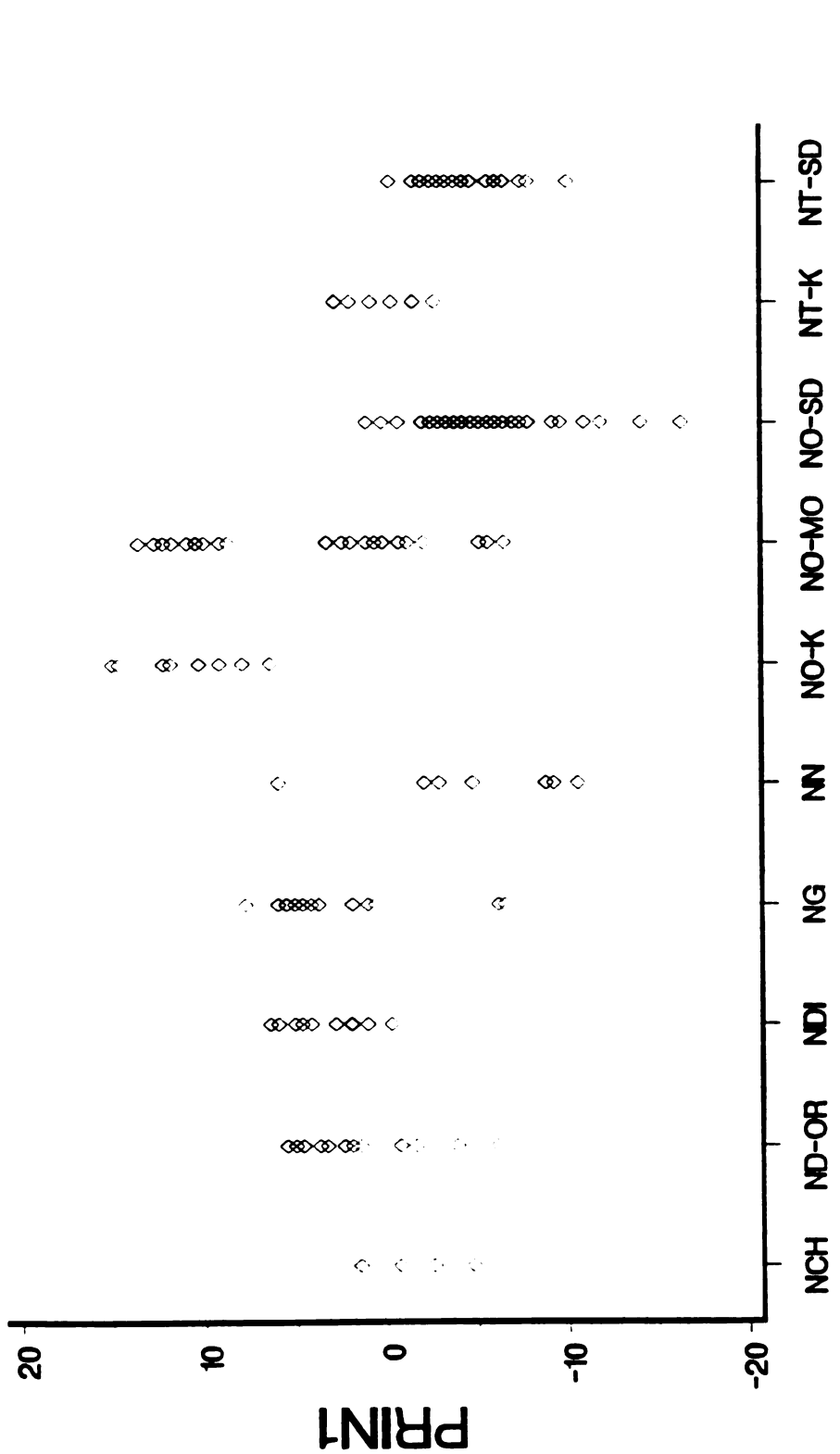


Figure 5.8 Principle components analysis of body size of mites based on the length and width of the sternal shield. The first principle component, which explains 92% of the variance, is plotted for mites taken from N. chilensis (NCH), N. defodiens from Oregon (ND-OR), N. didymus (NDI), N. guttula (NG), N. nigrita (NN), N. orbicollis (NO) from KBS (-K), Missouri (-MO) and South Dakota (-SD), and N. tomentosus (NT) from KBS (-K) and South Dakota (-SD).

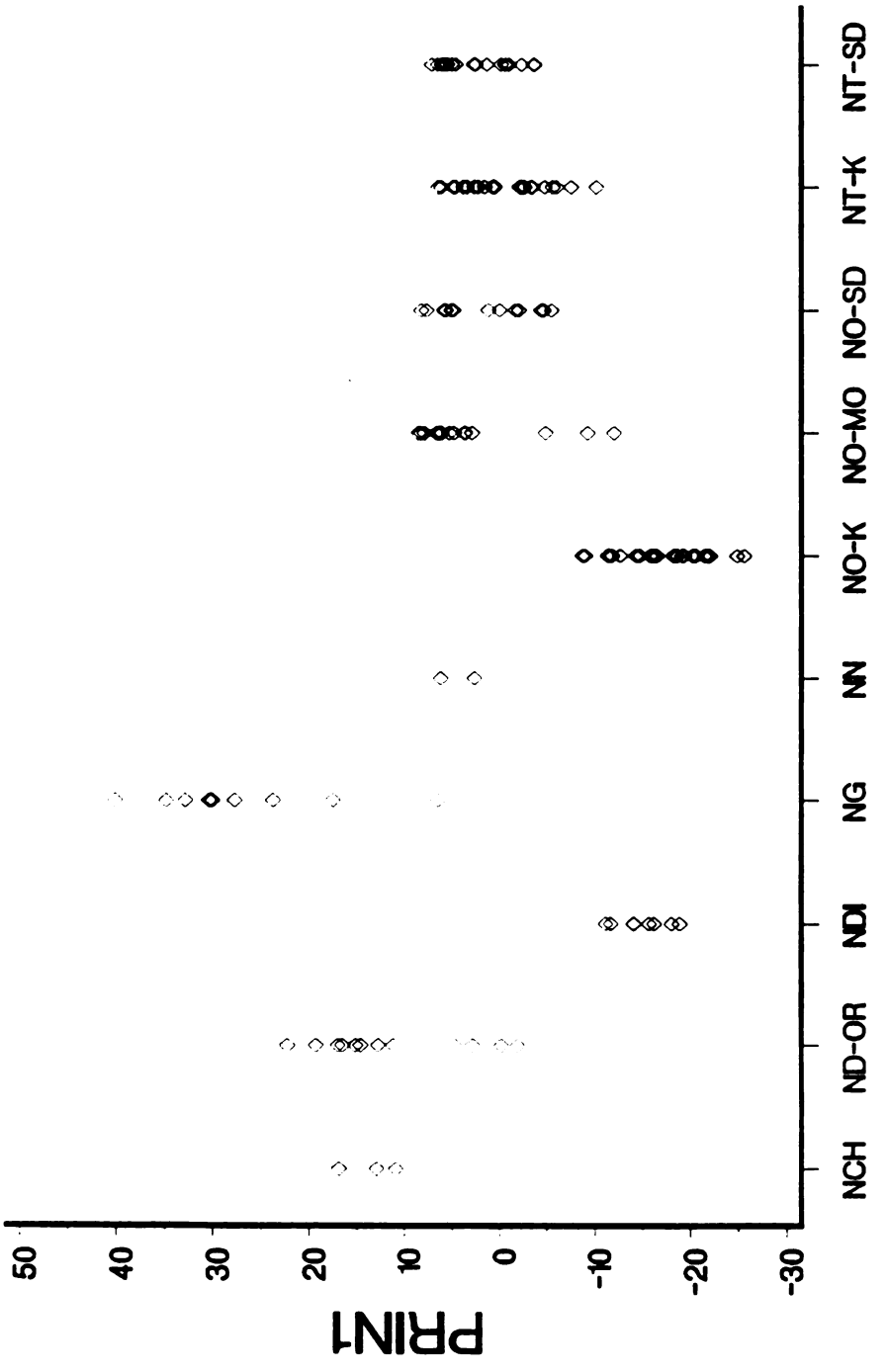


Figure 5.9 Principle components analysis of setal morphometry based on the lengths of 8 setae (i3, i5, i6, z2, z3, z4, s3 and s4). The first principle component, which explains 90% of the variance, is plotted for mites taken from N. chilensis (NCH), N. defodiens from Oregon (ND-OR), N. didymus (NDI), N. guttula (NG), N. nigrita (NN), N. orbicollis (NO) from KBS (-K), Missouri (-MO) and South Dakota (-SD), and N. tomentosus (NT) from KBS (-K) and South Dakota (-SD).

chilensis (Chile) and N. defodiens. These mites have very long setae, even in comparison with the tomentosus-specialist from Michigan. By contrast, mites from N. didymus (Peru) fall clearly into the orbicollis-specialist range, and are characterized by very small setal lengths.

DISCUSSION

A specific prediction of the both of my evolutionary hypotheses is that the northern range of the orbicollis-specialist in Michigan should coincide with the southern edge of N. defodiens distribution. While the data is not complete, it is compelling that on a coarse-grained scale this prediction is upheld. A more thorough study of this phenomenon would relate the relative densities of the specialists to the relative density of N. defodiens to N. orbicollis across the transect. Detailed behavioral tests across the transect may also help to discriminate between the two evolutionary hypotheses.

The data from sites outside Michigan is testimony to the wide variation in host use seen in these mites. The South Dakota data is particularly interesting, given the similarities in beetle species composition between these sites and my primary sites in Michigan. Mites from Farmingdale, South Dakota were generally of a single morphological type, similar to KBS tomentosus-specialists. Unlike at either Michigan site, these mites showed a less than complete preference for N. tomentosus over N. orbicollis. N. orbicollis carried moderate densities of these mites. This result was unexpected, since the beetle community contains the same members in

Farmingdale, South Dakota as at KBS, where the two specialist mites are very strict in beetle species use. Another unexpected result was the high preference of Hill City mites for N. tomentosus over N. defodiens. Since these tests were run in early July, presumably when N. defodiens are reproducing and N. tomentosus are not, one might expect mites to prefer the reproducing beetle species, as they do at UMBS. The similarities of the South Dakota sites in beetle species compositions to those at the Michigan sites may, however, conceal significant differences in the selective regime and/or population structure experienced by the mites. If beetle traits, such as degree of parental care, are themselves geographically variable, the species composition of a site may not by itself adequate describe the forces shaping the local evolution of specialization.

Interestingly, there was some evidence of morphological differentiation between mites with different behavioral phenotypes at the Farmingdale site in South Dakota. While this result does not prove that the patchy divergence scenario is correct in explaining the distributions of specialists in Michigan, it may indicate that divergence between ecological types can be found to different degrees at different sites. More detailed evidence that morphological differentiation is occurring in different ways at different sites would be strong evidence for the importance of local processes in the generation of diversity.

The comparison of P. carabi from a number of sites in North and South America provides an interesting, if crude, look at the range of variation in mite size and setal morphometry. The discovery of mites

with setae very similar to the KBS orbicollis-specialist on N. didymus from Peru is consistent with the hypothesis of an allopatric origin of divergence between the KBS specialists. As was outlined in Chapter 3, N. orbicollis is hypothesized to be most closely related to the South American Nicrophorus species (Peck and Anderson, 1985). The similarity of N. didymus mites to the orbicollis-specialist (Figure 5.9) is compelling given the hypothesized close evolutionary relationship between N. orbicollis and all the Central and South American Nicrophorus species. However, contrary to the prediction that the orbicollis-specialist morphotype might be common to all members of this "orbicollis-group," mites from N. chilensis from Chile resemble mites from the western North American Nicrophorus species in setal lengths (Figure 5.9). Thus it appears that not all South American species carry mites morphologically similar to the orbicollis-specialist. Interestingly, N. chilensis is hypothesized to be the most basal member of the orbicollis-group (Peck and Anderson, 1985). It seems unlikely, then, that the orbicollis-specialist represents a unique early invasion of North America, the phoretic mite accompanying the ancestor of the orbicollis-group. It would be interesting to examine mites from all Nicrophorus species in this group, who are themselves relatively unstudied, to explore the morphological variation in mites associated with many members of the species group. The resulting information, along with a complete biogeographical study of the N. orbicollis-mite association, would help greatly in discriminating between the allopatric and sympatric speciation hypotheses.

I should note that caution should be used in interpreting phenetic

similarity in one character as evidence of a close phylogenetic relationship, as I have done above. The possibility should not be overlooked that similarity in such a variable and perhaps selectively important character as setal length represents a convergent rather than a shared derived character state in KBS orbicollis-specialists and mites from Peru. In order to distinguish accurately between the allopatric and sympatric hypotheses, a proper phylogenetic analysis of the Poecilochirus genus, using many morphological characters, and an estimation of genetic affinities between mites of different behavioral, morphological and geographical types is required.

BIBLIOGRAPHY

BIBLIOGRAPHY

Anderson, R.S. 1982. Resource partitioning in the carrion beetle (Coleoptera:Silphidae) fauna of southern Ontario: ecological and evolutionary considerations. Can. J. Zool. 60:1314-1325.

Bartlett, J. 1987. Filial cannibalism in burying beetles. Behav. Ecol. Sociobiol. 21:179-183.

Bush, G.L. 1975. Modes of animal speciation. Ann. Rev. Ecol. Syst. 6:339-364.

Bush, G.L. and D.J. Howard. 1986. Allopatric and non-allopatric speciation; assumptions and evidence. in Karlin, S. and E. Nevo. Evolutionary processes and theory. Academic Press: Orlando, FL. pp. 355-365.

Cooreman, J. 1943. Note sur la Faune des Haute-Fagnes en Belgique 91). XI. Acariens (Parasitiformes) (2). Bull. Mus. hist. nat. Belg., Bruxelles, 19, No 63, 28 pp.

Darwin, C. 1859. On the origin of species (1st edition). Harvard University Press, Cambridge, MA.

Diehl, S.R. and G.L. Bush. 1989. The role of habitat preference in adaptation and speciation. (In press) In D. Otte and J. Endler (eds.), Speciation and its Consequences. Sinauer, Sunderland, MA.

Felsenstein, J. 1981. Skepticism toward Santa Rosalia, or why are there so few kinds of animals? Evolution 35:124-38.

Fox, L.R. and P.A. Morrow. 1981. Specialization: species property or local phenomenon? Science 211:887-93.

Futuyma, D.J. and G.C. Mayer, 1980. Non-allopatric speciation in animals. Syst. Zool. 29:254-271.

Futuyma, D.J. and G. Moreno, 1988. The evolution of ecological specialization. Ann. Rev. Ecol. System. 19:207-233.

Futuyma, D.J. and S.C. Peterson. 1985. Genetic variation in the use of resources by insects. Ann. Rev. Entomol. 30:217-38.

Hedrick, P.W., M.E. Ginevan, and E.P. Ewing. 1976. Genetic polymorphism in heterogeneous environments. Ann. Rev. Ecol. Syst. 7:1-32.

Hedrick, P.W. 1986. Genetic polymorophisms in heterogeneous environments: a decade later. Ann. Rev. Ecol. Syst. 17:535-566.

Hunter, P.E. and R.M.T. Rosario. 1988. Associations of Mesostigmata with other arthropods. Ann. Rev. Entomol. 33:393-417.

Hyatt, K. H. 1980. Mites of the subfamily Parasitinae (Mesostigmata: Parasitidae) in the British Isles. Bull. Br. Mus. Nat. Hist(Zool.). 38: 237-378.

Karg, W. 1971. Acari (Acarina), Milben Unterordnung Anactinochaeta (Parasitiformes) . Die freilebenden Gamasina (Gamasides), Raubmilben. Tierwelt Dtl. 59:1-475.

Kneissl, L. 1914. Uber Gamasoides carabi (Can.) Berl. und Neothrombium neglectum (Bruy.) Oudms. Zool. Anz. 45:33-35.

Korn, W. 1983. Zur Vergesellschaftung der Gamasidenarten Poecilochirus carabi G. u. R. Canestrini 1882 (=P. necrophori Vitzthum 1930), P. austroasiaticus Vitzthum 1930 and P. subterraneus Mueller 1859 mit Aaskaefern aus der Familie der Silphidae. Spixiana 6:251-279.

Levene, H. 1953. Genetic equilibrium when more than one ecological niche is available. Amer. Natur. 87:331-333.

Mather, K. 1955. Polymorphism as an outcome of disruptive selection. Evolution 9:52-61.

Maynard Smith, J. 1966. Sympatric speciation. Amer. Natur. 100:637-650.

Mayr, E. 1947. Ecological factors in speciation. Evolution 1:263-288.

Micherdzinski, W. 1969. Die Familie Parasitidae Oudemans 1901 (Acarina, Mesostigmata). Panstwowe Wydawnictwo Naukowe, Krakow, Poland.

Nei, M. and W.-H. Li, 1973. Linkage disequilibrium in subdivided populations. Genetics 75:213-219.

Neumann, K.-W. 1943. Die Lebensgeschichte der Káfermilbe Poecilochirus necrophori Vitzth. nebst Beshreibung aller Entwicklungsstufen. Zool. Anz. 142:1-21.

Peck, S.B. 1986. Nicrophorus (Silphidae) can use large carcasses for reproduction. Coleop. Bull. 40:44.

Peck, S.B. and R.S. Anderson, 1985. Taxonomy, phylogeny and biogeography of the carrion beetles of Latin America (Coleoptera:Silphidae). Quaest. Entomol. 21:247-317.

Peck, S.B. and M.M. Kaulbars. 1987. A synopsis of the distribution and bionomics of the carrion beetles (Coleoptera:Silphidae) of the conterminous United States. Proc. ent. Soc. Ont. 118:47-81.

Pimm, S.L. 1979. Sympatric speciation: a simulation model. Biol. J. Linn. Soc. 11: 131-139.

Pimm, S.L. and M.L. Rosenzweig, 1981. Competitors and habitat use. Oikos 37:1-6.

Rice, W.R. 1984. Disruptive selection on habitat preference and the evolution of reproductive isolation: a simulation study. Evolution 38:1251-60.

Rice, W.R. 1987. Speciation via habitat specialization: the evolution of reproductive isolation as a correlated character. Evol. Ecol. 1:301-314.

Springett, B.P. 1968. Aspects of the relationship between burying beetles, Necrophorus [sic] spp. and the mite, Poecilochirus necrophori, Vitz. J. Anim. Ecol. 37:417-424.

Turk, F.A. and S.M. Turk. 1952. IV-Studies of Acari.-7th Series: "Records and descriptions of mites new to the British fauna, together with short notes on the biology of sundry species." Ann. Mag. Nat. Hist., London, ser. 12, 5:475-506.

Vitzthum, H. v. 1930. Milben als Pestträger? Ein Beitrag zu den Untersuchungen der mandschurischen Preststudienkommission in Harbin.-Der "Acarologischen Beobachtungen" 16. Reihe. Zool. Jb., Syst. 60:381-428.

Wilson, D.S. 1982. Genetic polymorphism for carrier preference in a phoretic mite. Ann. Ent. Soc. Amer. 75:293-296.

Wilson, D.S. 1986. Adaptive indirect effects. in Diamond, J. and T.J. Case. Community Ecology. Harper and Row, New York, NY. pp. 437-444.

Wilson, D.S. and W.G. Knollenberg. 1987. Adaptive indirect effects: the fitness of burying beetles with and without their phoretic mites. Evol. Ecol. 1:139-159.

Wilson, D.S., W.G. Knollenberg and J. Fudge. 1984. Species packing and temperature dependent competition among burying beetles. Ecol. Ent. 9:205-216.

Wilson, D.S. and M. Turelli, 1986. Stable underdominance and the evolutionary invasion of empty niches. Am. Natur. 127:835-850.

Wise, G.U., M.K. Hennessy and R.C. Axtell, 1988. A new species of manure-inhabiting mite in the genus Poecilochirus (Acari: Mesostigmata: Parasitidae) predacious on house fly eggs and larvae. Ann. Entomol. Soc. Am. 81:209-224.