

Mating Systems of Cactophilic *Drosophila*

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The diversity observed in insect mating systems is of considerable interest to behavioral ecologists and geneticists (Blum and Blum, 1979; Krebs and Davies, 1978). In order to explain the forces which shape mating systems we need to understand the long term phylogenetic relationships and the ecology of the species being studied. Evolutionary relationships within the genus *Drosophila* have been extensively reconstructed (Patterson and Stone, 1952; Wheeler, 1981; Wasserman, 1982) and the ecology of a number of species, especially the cactophilics, has been the subject of intensive studies (Heed, 1978). Cactophilic species of *Drosophila* are predominantly from the very large *repleta* species group and the *nanoptera* species group. Information about their host plants, geographic ranges and nutritional requirements is contained in the chapters by Heed, Starmer, and Kircher in this volume.

The mating systems of cactophilic *Drosophila* species have recently been under investigation in my laboratory and have been found to differ in major ways from the mating systems of non-cactophilic *Drosophila*. The nature of these differences and the question of the evolutionary and ecological forces which may have created them are the topics to be discussed here. First, the mating systems of a number of cactophilic *Drosophila* species will be described. The species and their collection data are listed in Table I. Special emphasis will be placed on *D. mojavensis* for which the largest amount of experimental data is available. Then we will evaluate how the mating systems of cactophilic *Drosophila* differ from *Drosophila* species which utilize other types of resources,

TABLE I. *Species Studied, and Location and Date of Collection of Strains Used in Studies of Mating Systems*

<i>Species</i>	<i>Collection locality</i>	<i>Date</i>
<i>D. nigrospiracula</i>	Santa Rosa Mountains, Arizona	2/81
<i>D. mettleri</i>	Santa Rosa Mountains, Arizona	2/81
<i>D. mojavensis</i>	Santa Rosa Mountains, Arizona	2/80
<i>D. pachea</i>	Hermosillo, Sonora, Mexico	1/69
<i>D. arizonensis</i>	Tempe, Arizona	4/79
<i>D. hydei</i>	Tempe, Arizona	6/81
<i>D. nanoptera</i>	Zapotitlan, Puebla, Mexico	7/70

and suggest how these differences might be related to their cactophilic existence. Finally, the relationship between the mating systems and the ecology of various cactophilic species will be explored.

I Mating Systems of Cactophilic *Drosophila*

A. Age at Reproductive Maturity

In the course of a series of mating experiments performed in my laboratory and the laboratory of Dr. William Heed at the University of Arizona, it appeared that many cactophilic *Drosophila* species exhibit sexual dimorphism for the age at which reproductive maturity is reached (Cooper, 1964; Jefferson, 1977; Markow, 1981). Subsequently, this was measured for *D. mojavensis* in the laboratory (Table II). Ninety-seven percent of females were inseminated at three days of age, while many males did not inseminate females until they reached 8-10 days of age. Behavioural observations revealed that until males are about five days old they are not particularly interested in courting females and that young males who do court females do so much less persistently than do older males. These observations on sexual dimorphism in maturation have been generalized to include *D. nigrospiracula*, *D. mettleri* and *D. pachea* (Markow, Fogleman and Heed, unpublished).

TABLE II. Age at Reproductive Maturity for Males and Females of *D. mojavensis*

Sex	Age	Number mated	%
<i>Males</i>			
	3 days	43/156	27.6
	4 days	38/76	50.0
	5 days	62/82	75.6
	6 days	49/65	75.3
	7 days	63/77	81.8
	8 days	55/64	85.9
	9 days	74/81	91.3
	10 days	96/99	96.9
<i>Females</i>			
	1 day	2/47	4.2
	2 days	39/55	70.9
	3 days	58/60	96.7
	4 days	58/59	98.3

B. Incidence of Female Remating

A survey has been made of the frequency of female remating in a number of cactophilic *Drosophila* from the *nannoptera* and *repleta* species groups. These experiments were conducted in the laboratory by storing mated females apart from males and allowing them an opportunity to remate during a two hour observation period every morning for five days. The number of days required for females of each species to remate is shown in Figure 1(a). Females of *D. nigrospiracula* and *D. hydei* showed the shortest time until remating: nearly all females of these two species remated 24 hours after the first mating. In *D. nannoptera* some females required 48 hours to elapse before they would remate. The longest remating intervals were found for *D. pachea* and *D. mettleri* females. For comparison, similar data are presented for *D. melanogaster* females tested by the same procedures as employed with the cactophilic species.

In Figure 1(b), the proportion of females remating daily is presented. In *D. nigrospiracula* and *D. hydei* effectively all of the females remated daily. Approximately half of the *D. mojavensis* and *D. arizonensis* females remated daily. Daily remating was least frequent among females of *D. pachea*.

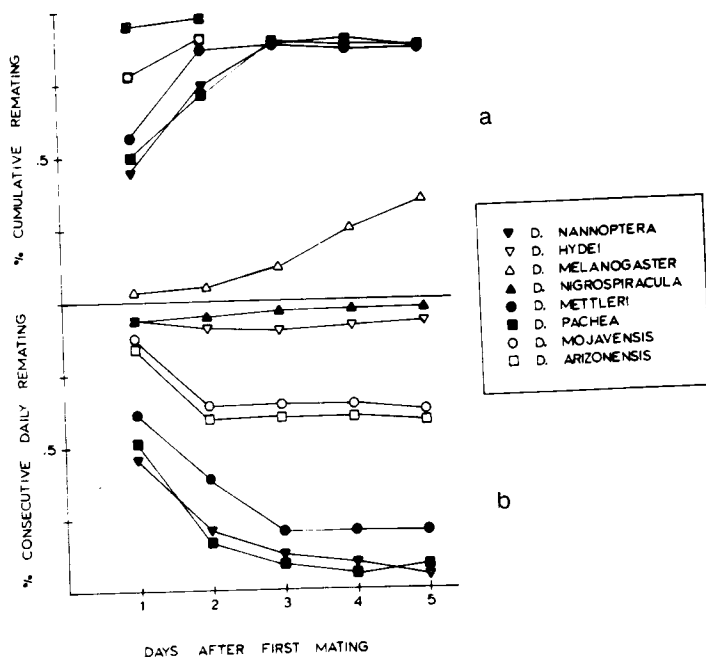


FIGURE 1. (a) The time until females of cactophilic species remate the first time. (b) Proportion of females remating daily for five days.

Since most *D. nigrospiracula* and *D. hydei* females remated at the 24 hour test, we determined how frequently they would remate if provided unlimited opportunities. Once females had mated they were transferred immediately to vials containing a new pair of virgin males and observed until they mated again. The number of times each female remated during a four hour observation period (8:00 a.m. - 12:00 noon) was scored. For *D. nigrospiracula*, the average number of matings was 3.89 ± 0.67 ($n = 41$) with several females mating five times in four hours. In *D. hydei* the average was 2.55 ± 0.94 ($n = 38$) matings. One *D. hydei* female mated four times. When females of either species are dissected after multiple matings, the ventral receptacles contain a number of discrete clusters of sperm equal to the observed number of matings. Within 12 hours, the sperm from all matings appear to be mixed within the receptacle. The influence of multiple matings in one day on daily mating incidence was investigated in

D. nigrospiracula, the species showing the highest frequency of female remating. Females were mated twice on the first day and then the number remating twice on consecutive days was examined. On the first day after mating twice, 87% (27/31) of the females remated two more times. On the next day 80% (24/30, one female escaped) remated twice. Three days later 77% (23/30) remated twice and on the last day 60% (18/30) still engaged in a double mating. None of the other species exhibited female remating during the same morning, with the exception of *D. mettleri* where three different females (out of 81) were observed to remate during the observation period.

C. *Effect of Remating on Female Fitness - Laboratory Investigations with D. mojavensis*

Most *D. mojavensis* females remate a second time 24 hours after their first copulation, as described above. The remainder remate at 48 hours when given a second opportunity. Figure 2 shows the daily productivity rates for two weeks for females that mated once, twice (remated after 24 hours), twice (remated after 48 hours) and females continuously paired with males. In females that only mated once, productivity fell dramatically from the fourth day. Females which mated twice laid eggs longer when the second mating was 48 hours after the first. The continuously paired females maintained their productivity the longest. That this is not merely a function of the presence of males has been established by experiments in which males were present but separated from females by a mesh partition. In terms of total productivity, females paired continually produce the most offspring (Mean offspring number \bar{x} = 380). A second mating did not increase overall productivity unless it occurred 48 hours after the first (\bar{x} = 82). Single mated females gave the same number of offspring (\bar{x} = 51) as twice mated females who remated after only 24 hours (\bar{x} = 53). Inspection of the ventral receptacles two weeks after a single mating reveals large quantities of motile sperm. That the viability of these sperm is still high is suggested by the fact that egg to adult survival does not decline with time after mating. Out of 100 eggs deposited by females one day after mating 91 adult flies were obtained. Eggs collected from single mated females ten days after mating had an adult survival rate of 92%.

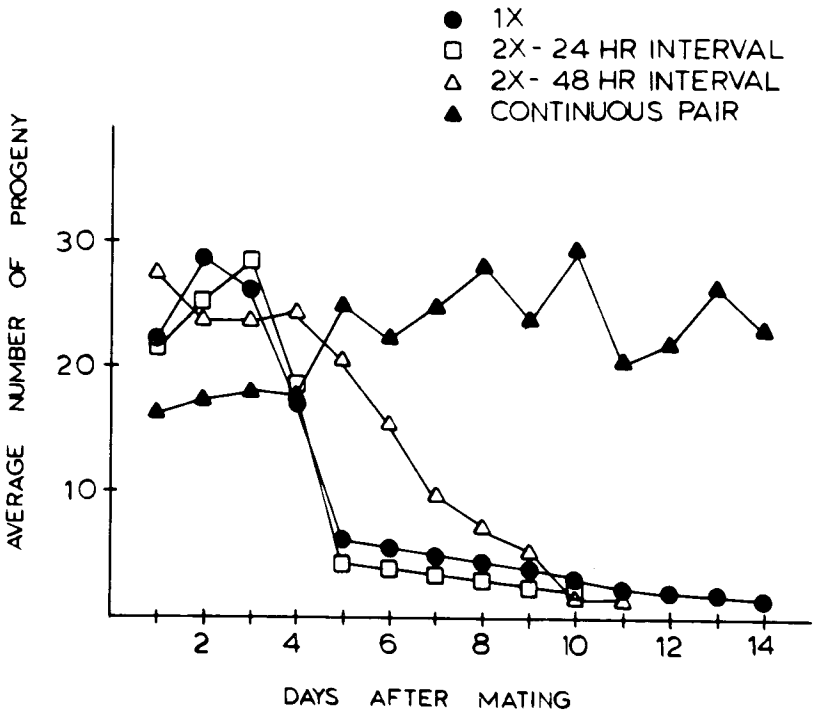


FIGURE 2. Daily productivity (adult progeny) for *D. mojavensis* females for two weeks. Open squares = inseminated once, solid circles = inseminated twice 24 hours apart, open triangles = inseminated twice 48 hours apart, closed triangles = continuously housed with males.

D. Effect of Remating on Male Fitness - Laboratory Studies with D. mojavensis

Two aspects of female remating for male reproductive biology were considered. In *D. melanogaster*, males exhibit a dramatic reduction in fertility following the third successive mating. Due to the overall increase in mating frequency by cactophilic species, we became curious about the fertility of multiply-mated male *D. mojavensis*. Males were presented with virgin females until they had mated seven times in succession during a two hour observation period. Males showed no decrease in mating propensity over the seven matings. Females were saved and their offspring counted. The

offspring number did not decrease by the seventh mating (Fig. 3). How *D. mojavensis* males maintain their fertility level across so many matings might become clear from study of factors such as quantity of sperm and the nature of ejaculate components transferred at each mating.

The other problem that multiple female mating poses for males is inter-ejaculate competition. Since females store sperm, intrasexual selection can occur within the female reproductive tract. We were able to study the sperm utilization pattern in *D. mojavensis* by employing a recessive mutation causing dark eye color which arose spontaneously in the laboratory. Dark-eyed females were mated either to dark-eyed males first and then to wild-type males or to wild-type

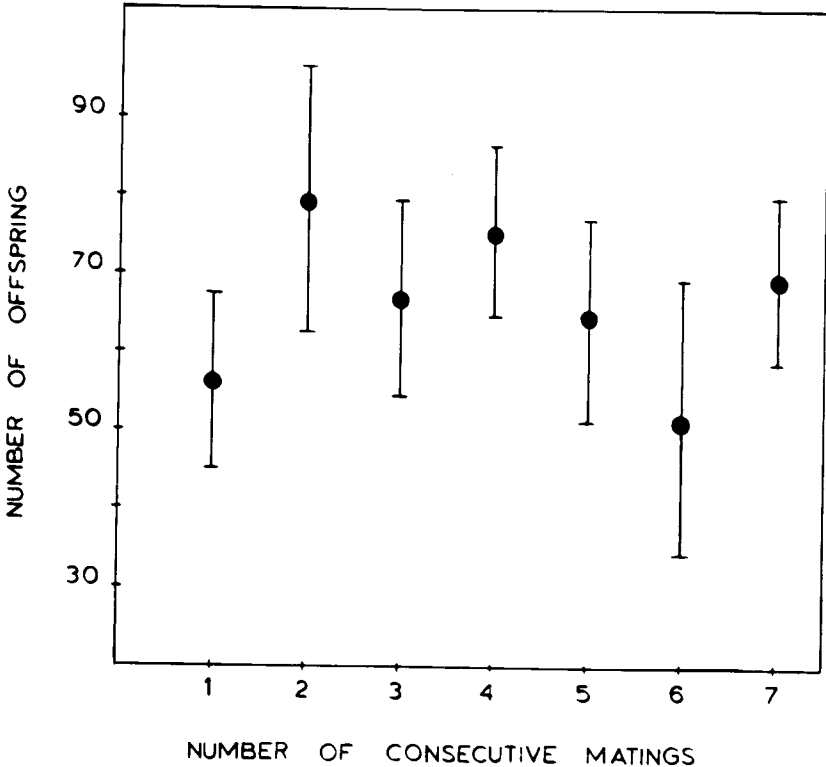


FIGURE 3. Average productivity of males on their first through seventh consecutive matings.

males first and then to dark-eyed males. The relative number of dark-eyed and wild-type progeny were then used in calculating the proportion of offspring sired by the second male (P_2). The second mating occurred either 24 or 48 hours after the first. The relative contributions of the first and second mating are shown in Table III. A 2 X 2 factorial analysis of variance showed significant differences between the 24 and 48 hour time intervals. There was no effect of genotype of the second male or significant interaction between remating interval and genotype (Table IV).

While early remating may allow females to maintain their oviposition rates, the temporal change in P_2 values suggests an advantage exists for males that are able to assure that longer intervals have elapsed before they mate with a non-virgin. Such assurance would be afforded to males that are able to assess the time since a female last mated and in

TABLE III. P_2 Values in Experiments where Rematings were 24 hours Apart and 48 hours Apart. The Genotypes of the First and Second Mates were Alternated for each Remating Interval

Remating interval	Genotype of second male	P_2 (%)
24 hours	wild type	64.85 ± 2.48
	dark eye	68.17 ± 1.72
48 hours	wild type	97.63 ± 3.73
	dark eye	96.91 ± 4.78

TABLE IV. A 2 X 2 Factorial Analysis of Variance on Arc-sin Transformed P_2 Values

Source of variation	D.f.	Sum of squares	Mean squares	F
Total	15	5373.9		
Genotype	1	3.7	3.7	0.48
Time interval	1	5263.9	5263.9	684.51 ^a
Genotype x time	1	14.1	14.1	1.83
Error	12	92.2	7.7	

^a $P < 0.001$

doing so, increase the prospective success of their own sperm prior to investing time and energy in courtship. A series of male courtship choice experiments (Markow, 1981) was conducted to examine the possibility of pre-courtship discrimination. Individual virgin males, placed in a chamber with a virgin female and a mated female were observed until they courted a female. The proportion of the males that first courted a virgin instead of a non-virgin is seen in Table V. The data clearly show that males discriminate between females prior to investing in courtship. The preference for courting a virgin female does not disappear until the time corresponding to the male's ability to fertilize over 98% of subsequent eggs.

At the time that sperm utilization in *D. mojavensis* was investigated, the incredibly high remating frequency in female *D. nigrospiracula* and *D. hydei* had not been discovered. Obviously these species, especially *D. nigrospiracula* with its unique male-male interactions, present an interesting problem for paternity assurance and their sperm utilization patterns need to be investigated.

E. Inbreeding and Sterility in D. mojavensis

A potential relationship between remating and inbreeding avoidance is suggested by the outcomes of pair matings between related and between unrelated individuals. A variety of studies have required that pair matings be done for various desert species of *Drosophila*. These experiments frequently encountered difficulties because sibmated lines often failed to reproduce. An analysis of a similar situation in *D. melanogaster* showed that inbred lines failed because males are not stimulated to court closely related females (Averhoff and Richardson, 1974). The possibility that loss

TABLE V. *The Outcome of Male Choice Experiments where Single Males were Offered Two Females, One Virgin and One Having Recently Mated*

Interval since mated female had mated with other male	First female courted	
	Mated	Virgin
2 hours	2	46
24 hours	6	39
48 hours	18	28
96 hours	29	26

of fertility in *D. mojavensis* was occurring by a similar mechanism was examined in inbreeding experiments in which flies were sibmated.

A total of 22 sibmated lines was started, each from the descendants of individual wild caught females. In the second generation 10 pairs of progeny from a single female were selected and placed in vials. The number producing progeny out of the 10 pairs for each of the 22 lines was recorded. Each subsequent generation was begun by taking 10 pairs from one of the vials of a given line. This inbreeding scheme was carried out for five generations. At the second generation, seven of the 22 lines failed to reproduce. In each case, all 10 vials from a non-reproductive line were affected. A complete absence of eggs suggested that females be dissected and examined for evidence of insemination. Five of the 10 females from each of seven lines were dissected, and the ventral receptacles of each female were found to be filled with motile sperm. The other five females were then mated to unrelated males from other non-reproductive lines. All females but one subsequently produced viable offspring indicating that both males and females were fully fertile with unrelated mates.

In each subsequent generation, about half of the inbred lines became non-reproductive. In every case, all 10 of the pairs failed to oviposit, although females did contain motile sperm and both males and females could reproduce when re-paired with unrelated individuals from other lines. The only apparent deterrent to oviposition was the degree of relatedness of the pair. A factor or factors controlling reproduction may be segregating.

Males of *D. melanogaster* transfer an ejaculate component which is required to stimulate oviposition (Garcia-Bellido, 1964; Merle, 1968). The observations in *D. mojavensis* could be explained by the inability of such a component to trigger egg laying when the male is too closely related to the female, i.e., a sort of "self sterility" system. If such a system were operating it would tend to favor outbreeding and the maintenance of genetic variation in a population.

II Relationship of Mating Systems to the Ecology of Cactophilic *Drosophila*

In comparison with other species groups, the cactophilic *Drosophila* and their relatives in the *repleta* and *nannopectera* groups are characterized by sexual dimorphism for age at reproductive maturity and frequent female remating. In

non-cactophilic species such as *D. pseudoobscura* (Pruzan, 1976), *D. simulans* (Markow, unpublished) and *D. melanogaster* (Pyle and Gromko, 1978) where females eventually remate, the average remating latency is a number of days. While the cactophilic *Drosophila* clearly remate more frequently than other species, considerable variability exists among them for this behavior. Thus two questions can be asked. First, why do frequent female remating and delayed male maturity occur in the cactophilic species? Second, what factors control the variability in remating frequency among these species?

In answer to the first question, the relatives of cactophilic species also exhibit female remating. Therefore it is possible that this trait existed among ancestral *repleta* and *nannoptera* species and served as a preadaptation for their utilization of cactus as host plants. An alternative hypothesis could be that some other trait or traits, physiological perhaps, served as the initial preadaptation for being cactophilic. The possibility that the subgenus *Drosophila* as a whole is characterized by more frequent female remating than the subgenus *Sophophora* is one that remains to be explored. I would like to suggest the hypothesis that the mating systems of the cactophilic *Drosophila* are related to the characteristics of the breeding sites provided by necrotic cactus and suggest certain ways to examine this hypothesis. Rot pockets are usually patchily distributed and each necrosis undergoes a succession of stages. The actual time during which a rot is attractive for oviposition appears to be a highly transitory one (W.B. Heed, pers. comm.). In order for females to take advantage of optimal oviposition substrates, it would appear that they need a ready supply of sperm and eggs. Frequent remating should assure an available sperm supply. But it may also serve to increase egg production (Fig. 2). The possibility that females remate frequently to obtain some component(s) from the ejaculate must be considered. The value of nutrients transferred to females at mating has been nicely documented for the Lepidoptera, and is currently being studied in *Drosophila* in my laboratory (Boggs and Gilbert, 1979; Boggs, 1981).

Another problem apparently confronting these flies is inbreeding. If rots are few and far between or if populations crash in the heat of the summer, populations of related individuals would tend to build up around particular necroses. Laboratory observations suggest that none of the cactophilic species are very tolerant of inbreeding (Cooper, 1964; Jefferson, 1977). One situation that would seem to reduce matings between sibs is the age differential between males

and females for reproductive maturation, which is seen in all cactophilic species that have been mated in the laboratory. Further, frequent remating increases the chances of eventually copulating with an unrelated individual. Superimposed upon this might be a mechanism whereby females do not release eggs when inseminated by too close a relative, a sort of "self sterility" situation. The existence of behavioral and physiological mechanisms for preventing inbreeding is also testable and the data from *D. mojavensis* suggests that the other cactophilic species should be studied under inbreeding regimes. The relationship between the mating status of males and females and the timing of migration to a new rot is an unknown which bears on the question of gene flow and the genetic structure of the populations. Furthermore, self sterility mechanisms have been proposed to be of importance in the evolution of mate recognition systems during speciation (Templeton, 1980).

The evolutionary significance of the considerable variability among cactophilic species in the incidence of remating is not clear. Causes may be sought in both mechanistic and evolutionary terms. For example, in certain *repleta* group species from the *mulleri* complex, copulation is followed by the appearance of an insemination reaction, a mass of opaque, non-cellular material that fills the female's uterus and lasts up to 10 hours (Patterson, 1947). The presence of the reaction mass may temporarily prevent remating as well as oviposition in those species which exhibit it. Of the species included in the present study, only *D. mojavensis* and *D. arizonensis* show a reaction mass. Females of these two species do not ordinarily remate for 24 hours. On the other hand, the two *repleta* species which multiply mate in a given morning, *D. nigrospiracula* and *D. hydei*, do not form this mass. Thus the presence or absence of a reaction mass could be one factor underlying the remating propensity of females of certain species. Another factor proposed by Walker (1980) to influence female remating is the size and shape of the female sperm storage organ. Longer storage organs as opposed to spherical ones were suggested to predispose females to more frequent mating. The *repleta* species included in the present study all store their sperm in the ventral receptacle and not in the spermathecae, and all have very long receptacles as compared with other species groups. Females of *D. hydei* have the longest receptacles in the genus (Wasserman, 1960) and together with *D. nigrospiracula* which also has a long receptacle, show the highest incidence of remating ever reported for *Drosophila*. In all of the *repleta* group species examined so far, sperm are found in the

proximal portion of the ventral receptacle immediately after mating. Within 12 to 24 hours, they are distally located and the proximal portion is relatively empty. Perhaps the availability of space in the proximal ventral receptacle governs whether or not a remating will occur. A long ventral receptacle may simply require a number of copulations in order to fill with sperm. The ventral receptacle does appear to be innervated and may thus provide one level of control of female receptivity (Miller, 1950).

Genetic and physiological control of the timing of remating has been most extensively investigated in *D. melanogaster*. Manning (1967) suggested that the act of copulation itself causes a mechanical block to remating that lasts up to 24 hours in this species. After that time, the inclination of a female to remate is assumed to depend upon the degree to which the supply of motile sperm in her ventral receptacle has been reduced. Reduction in the supply of motile sperm could result from their being used up during oviposition and/or from factors in the ejaculate or female reproductive tract which influence sperm viability (see Gromko *et al.*, 1981 for an extensive review). Obviously in *D. hydei* and *D. nigrospiracula* no such 24 hour block exists. In all of the cactophilic *Drosophila* species examined in my laboratory, the utilization of sperm by oviposition appears to have no detectable effect on the timing of remating. Females of any given cactophilic species appear to remate just as quickly whether they have been allowed to use up sperm by ovipositing or not.

The role of ecology in the differences in mating systems between various cactophilic species is more speculative at this time. One obvious variable is the nature of the breeding sites provided by the preferred host plant of each species. Saguaro and cardón, the hosts of *nigrospiracula*, are much larger than organ pipe and agria, which are utilized primarily by *D. mojavensis*, and senita, utilized by *D. pachea*. The opuntias provide a different substrate entirely from the columnar cacti. The size, shape and moisture content of the cactus undoubtedly influence the features of a rot and the breeding site it provides. The presence of other species which might be competing for the same substrate may also influence mating strategies. *D. pachea* has few problems with competitors since most species are prevented from using senita by the toxic alkaloids in this cactus (Heed, 1978). But *D. nigrospiracula* may have evolved its intense mating activity as a means of keeping other species from utilizing its host plant.

In summary, certain mating system features are apparently characteristic of the cactophilic flies in the *repleta* and