Host Shifts of Chymomyza Amoena (Diptera: Drosophilidae)

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ABSTRACT: *Chymomyza amoena* (Loew) is a domestic species that has expanded its host niche to breed and overwinter in apples in Michigan and the mid-South. Populations remain multivoltine throughout the latitudes investigated, East Jordan, Mich. (45.10°N) to Eden, N.C. (36.29°N). A new generation can be produced within a month during the breeding season. Populations remain polymorphic for developmental time. The polymorphism has been investigated in apples, but lengthy emergence from crabapples indicates it predated the invasion of apples. Populations overwinter in the larval stage in Michigan; the pupal stage may be the overwintering stage in milder climates or it may be entered early in spring. Populations within Michigan and between Michigan, Missouri and the mid-South have been interfertile. This species has a longer prefertile period than *Drosophila melanogaster*. Comparisons of the oviposition rate of pairs captured in nature with laboratory crosses involving aged and nonaged parents indicate that females in nature do not approach males until past their prefertile period. Multiple use of endemic host plants for breeding has been investigated in mid-Michigan. Females emerging from black walnut husks prefer to oviposit on softened, overwintered native crabapples; females emerging from crabapples early in summer can breed in immature, unripe fallen domestic apples or plums. Females rely on pest species to attack firm substrates first and oviposit in scars, holes or directly in frass when available. Both males and females are attracted to sites where other *C. amoena* have oviposited. Later in summer females will also oviposit on unfallen fruits. Larvae pupate within the substrate or in soil. Studies at Mt. Lake Biological Station where other chymomyzid species occur show that males will court inter- or intraspecifically. Capture-in-the-air is an alternate mating system in this species to the assault-type mating predominant in this, other drosophilids and other Diptera. The simple courtship and other behaviors are described. Population size is small and, to date, there is no evidence of sympatric or allopatric speciation.

INTRODUCTION

Chymomyza amoena is one of the few species in its genus, within the Drosophilidae, which occurs in domestic settings (Band and Band, 1980, 1982a,b, 1983, 1984, 1987). Sturtevant (1921) reported that it had been bred from black walnut husks *Juglans nigra*, butternut husks *J. cinerea* and acorns *Quercus* spp. (Wheeler, 1952; Throckmorton, 1975; Grimaldi, 1986). Sturtevant (1921) stated that he and Metz bred *C. amoena* from apples. Chymomyzids are considered to be woodland species and sap feeders. Steyskal (1952) collected *C. amoena* on cut ends of trees. Sabath (1974) and Sabath and Jones (1973) included it among the broad-niched Indiana drosophilids. They collected adults in gardens and varied woodland settings but did not investigate substrate use.

Host expansion by *Chymomyza amoena* into apples raises the question of founder effects, small population size and speciation (Mayr, 1963, 1982; Templeton, 1980a,b, 1981, 1982; Bush, 1975a,b, 1982). Band (1979, 1985) found it overwintering in apples in Michigan and in Virginia, indicating a relatively widespread invasion of the apple niche, although nuts and nut husks continue to be used in Michigan also (Band and Band, 1982a,b, 1984, 1987). Therefore, the potential exists for sympatric and allopatric speciation.

Seasonal studies carried out for cold hardiness changes between summer and winter larvae revealed that this species can be found in immature, firm fallen apples in summer as well as firm substrates like native crabapples *Malus coronaria* and walnut husks in winter (Band and Band, 1984). Drosophilids require damaged surfaces for oviposition

(Carson and Heed, 1983) although the fig-breeding lissocephalids (the most primitive drosophilids) use unripe unfallen figs via bract oviposition and entrance into the fig cavity (Lachaise, 1977; Lachaise and Tsacas, 1983).

Malus coronaria, *Juglans nigra* and various oak species are endemic to North America and native to Michigan (Barnes and Wagner, 1985). *Chymomyza amoena* is also thought to be endemic to North America. Domestic (imported) apples were brought to this country in the 1600s and carried westward by Indians and settlers alike. Professor Davis of Michigan Agricultural College, now Michigan State University, recorded *Chymomyza amoena* emerging from apples along with codling moth *Cydia pomonella* in the East Lansing area in 1891 (Band and Band, 1982b). These results raise specific questions about *C. amoena* that may also have a bearing on the chymomyzids in general. Most species are not amenable to laboratory study.

We have bred *Chymomyza amoena* from apples collected as far N as East Jordan, Mich. (lat 45.10°N) (Band, 1979; Band and Band, 1980, 1982a,b) and as far S as Eden, N.C. (lat 36.29°N) (Band, 1985). Thus, the widespread invasion of apples over this substantial geographical range would likely entail adaptation to various lengths of growing season and to varied exposure to apples as a breeding substrate. We therefore investigated Virginia *C. amoena* populations during 2 summers at Mt. Lake Biological Station to provide more information on behavior, geographical variation, small population size and the possibility of allopatric speciation.

In this paper we investigated the following questions:

1. How do females oviposit and larvae gain entrance into firm substrates?
2. Were genetic changes involved in host expansion?
3. Does small population size promote speciation?
4. Is developmental polymorphism recent or does it predate apple-invasion?
5. Are there geographical differences between populations?

The work was done in northern lower Michigan (1978-1981), mid-Michigan (1979-1986) and the mid-South (1984-1986).

MATERIALS AND METHODS

The research was divided into three phases: 1978-1981, 1981-1984, and post-1984. Phase one concentrated on (1) verifying extensive apple use in Michigan; (2) duplicating in the laboratory the long emergence period from apple collections in summer; (3) devising a medium that would support growth and reproduction in the laboratory, and (4) determining that Michigan populations were not reproductively isolated from one another or from Missouri (St. Louis, lat 38.38°N) populations mailed to East Lansing by the late Harrison Stalker. Observations were also made on the natural population at East Jordan.

Phase two centered on (1) multiple host usage in the greater Lansing-East Lansing area (lat 42.43°N), and lack of evidence for genetic changes mediating host shifts; (2) comparison of captured pairs with laboratory data on oviposition; (3) use of unripe fallen apples in June and early July, and (4) the discovery that females later oviposited on parasitized fruits on trees.

Phase three compared behavior of *Chymomyza amoena* in Virginia with that in Michigan. This includes emergence from spring-overwintered and new season's apples in summer, oviposition on fallen and unfallen fruits, and pupation in substrate versus soil.

STUDY SITES

Northern Lower Michigan.—The data from northern lower Michigan come from *Chymomyza amoena* reared from fallen apples from a group of old apple trees at East Jordan. Overwintered apples were collected in spring (March to May). The new season's apples began to form in June and to fall in July. Summer collections were made in July and August, autumn collections in September and October. All apples were transported to the laboratory, pooled into 1-3 containers per collection, maintained at room temperature (22 C) until a week after the last *C. amoena* emerged (*see* Heed, 1968) or cultures became too mite-infested and were discarded. Initial emergence dates from apples in autumn do not differ from initial emergence dates in summer and demonstrate that a complete generation is possible during the breeding season in that area. Multiple laboratory populations were established from the East Jordan site (EJ), labeled also by month and year to identify collecting date.

Other Lower Michigan sites. — *Chymomyza amoena* were also obtained from apples in an abandoned orchard near Grand Rapids (W Michigan, lat 42.58 °N), commercial orchards and Rose Lake Wildlife Station near East Lansing (lat 42.44 °N) in mid-Michigan. Populations were established in the laboratory and designated GR (Grand Rapids) and RL (Rose Lake). Collections at the commercial orchards and a farm N of Lansing, which had been sprayed, were made in winter or in March.

Mid-Michigan. — For continuous studies in the mid-Michigan area, certain sites have provided substrate-infested *Chymomyza amoena* yearly. Site A: A farm W of Lansing with adjacent apple and black walnut trees and other black walnut trees on the property. Site B: An East Lansing neighborhood having a variety of fruit trees. Sites C and D: farms E of East Lansing. Site D has black walnut and oak trees and an occasional producing apple tree. All native crabapples came from a thicket 22 km from East Lansing near Williamston. This thicket, of unknown age, was in existence until 1987; Dr. George Parmalee of Michigan State University deposited specimens from it in the Beal-Darlington Herbarium in 1953. Unripe fallen plums (*Prunus* spp.) were then investigated at sites B and C.

In mid-Michigan, summer is the traditional June, July and August; autumn is September, October and November; winter is December, January and February. The breeding season begins in May.

Mid-south sites. — Overwintered apples were collected at a noncommercial orchard near Danville, Va. (lat 36.35 °N) in April 1984 and again in March 1986. *Chymomyza amoena* were observed displaying on fallen apples in a garden at Eden, N.C. (lat 36.29 °N) in June 1984. Two collections of apples were made in June. Studies were begun at Mt. Lake Biological Station (MLBS) in the Allegheny Mountains near Blacksburg, Va. in mid-July 1985. Collecting sites were established along Rt. 700 to the Station and near Mt. Lake Hotel; collections were also made at the orchard near Danville. In 1986 work began in June. Collections were made at an abandoned orchard near Blacksburg (lat 37.14 °N) and at the Station. Apples were also obtained from a farm at Pamplin (near Lynchburg, lat 37.24 °N) in June and again in August and from a neighborhood in Danville.

EXPERIMENTS AND OBSERVATIONS

Substrate emergence, interfertility and behavioral studies comprise the three broad research categories. Michigan and the 1984 mid-South studies provided the basis for the work at MLBS. Data on other insects emerging from substrates were also obtained.

1. *Duration of emergence.* — The handling of apples has been described. The procedure was applied to all substrates from all localities in Michigan. Number of apples collected at East Jordan was recorded in most but not all cases, 1978-1981. Numbers emerging and dates of emergence were recorded per collection. Emergence records from individual pieces of fruit were not obtained until 1985. Emergence from overwintered apples in lower Michigan was determined in 1980 and 1981.

In early July 1981 at site A, 22 unripe fallen apples were gathered on which adults were displaying (wing-waving). When flies began emerging in the laboratory, adults were soon observed again on riper fallen apples at this same site. Fifteen were gathered.

Emergence from softened, overwintered native crabapples was determined in May 1982 after 27 were found to have 45 newly oviposited *Chymomyza amoena* eggs.

Numbers and emergence duration from the winter-collected walnuts at site A were determined in January 1983. Walnuts were sampled also at this same site in November

1983 and from site D, E of East Lansing in November 1984 when there was a heavy invasion of this substrate.

Duration of emergence from plums at B was determined in June 1987 and from fallen apples from adjacent trees when females began ovipositing on them. Plums at C were also collected.

All overwintered apples from the mid-South in April 1984 and March 1986 were inspected and those with eggs and larvae were retained for transport back to East Lansing. In 1986 emergence was completed before return to East Lansing.

Unripe apples in June 1984 from Eden, N.C., were similarly inspected and those with *Chymomyza amoena* eggs and larvae retained for transport to East Lansing. Data yield initial emergence dates from spring-overwintered and from summer-collected apples in the mid-South to compare with those for *C. amoena* in northern lower and mid-Michigan. Emergence of other insect species provides information on geographical differences on niche sharing, if any.

2. *Duplication of the lengthy emergence period from apples.*—To duplicate the emergence period from summer-collected apples at East Jordan, F_1s from a pair emerging from an October 1978 collection were serially grouped as they emerged and transferred to fresh apple quarters for mating. The first set contained 10 flies emerging over days 36-39; the second set consisted of two sets of 10 flies each emerging over days 42-46; the final set comprised four flies emerging over days 47-49. Apple pieces were inspected every 2 days until oviposition began. Pieces on which eggs had been deposited were transferred to jars containing moist paper toweling. Numbers and dates of emergence were recorded.

3. *Intrapopulation prefertile period, interpopulation, intersubstrate and interstate fertility.*—Two small populations, collected in August and November at the same St. Louis, Mo., site, were received in November 1980 and transferred to the artificial medium used for Michigan flies. This is a protein-containing medium (Band, 1981d, 1984) based on that devised for the Hawaiian *Drosophila* (Wheeler and Clayton, 1965). Crosses within Michigan populations (EJ, GR and RL) and between MI and MO populations were set up in two ways. In the first set, males and females were mated upon eclosion. In the second set, males and females were collected over a week before crosses were made, effectively aging the flies. In the first set, length of the prefertile period prior to the appearance of eggs and length of the period between oviposition and hatching were determined. In the second set, length of the time between setting the cultures and oviposition was determined. F_1s were reared and fertility determined by the production of an F_2 generation. Crosses were set typically with three males and three females. Small mass matings to determine interpopulation fertility within and between states and countries have been used by Dobzhansky (1937), Patterson (1954) and Throckmorton (1982).

The first set of crosses involving nonaged parents employed the November Missouri collection, the set involving aged parents employed the August Missouri collection; crosses between the August and November Missouri set established interfertility and indicated that the population in Missouri has no obligate diapause. Michigan flies in both experiments were emergees of September or October 1980 apple collections, except for the GR 1979 population only. However, when experiments with aged parents were set, reciprocal crosses were made also between nonaged parents from different EJ collections: April 1979, August and October 1980 stocks.

Crosses were also made in 1984 between the North Carolina population and an East Lansing population newly isolated from apples. Nonaged parents were used. Length of the period prior to the appearance of eggs was compared with that of intrapopulation NC crosses and both compared with previous interstate and intrapopulation crosses.

To verify interfertility of Michigan adults emerging from the different substrates, reciprocal crosses were made between emergees from native crabapples in May 1982 and apple-derived stock. Similarly, reciprocal crosses were made between walnut emergees in January 1983 and apple-derived stock. Again, nonaged parents were used. Interstate fertility was assessed by reciprocal crosses of the North Carolina *Chymomyza amoena* stock with 1984 Michigan emergees from site D walnuts.

4. *Adult behavior, oviposition, emergence and reproduction of captured pairs.*—Michigan and Missouri populations were compared for mating behavior and for female avoidance of 'courting' males. Natural population behavior was studied at East Jordan, Mich. in August 1979, and also monitored at sites A and B in mid-Michigan.

Adults collected on seven fruits at site A in 1981 were males, on six fruits the next day numbered three males and two females (one escaped). These were transferred to a population bottle containing medium + apple piece and time to oviposition recorded. A pair captured at site B in 1982 was placed on apple only; time to oviposition was recorded.

In Michigan, small immature apples on which adults were displaying in 1981 had *Chymomyza amoena* eggs in crevasses, scars (plum curculio *Conotrachelus nenuphar*) or holes made by pest larvae. Location and number of eggs were recorded for the 17 immature apples gathered at site B in June 1982 and for 22 ripe fallen apples collected 31 July at site C in 1982. Pears with and without frass were pulled from a tree at site B and location of eggs recorded in late summer 1982. In July 1983 immature fallen frassy apples at sites A and B were gathered and location of *C. amoena* eggs recorded. In mid-July 1984 all apples on the ground at site B were gathered and total number of eggs vs. numbers in frass compared. A windstorm blew down many developing apples from the tree at site B on 31 May 1985. On 13 June 1985 all 179 apples on the ground were gathered, scored for the presence of *C. amoena* eggs and dissected for the presence of frass internally.

Drosophilids may or may not leave the substrate at pupation (Carson *et al.*, 1970; Grossfield, 1978). Three of 17 apples from site B in 1982 were retained and dissected 2 weeks later for pupal location. A similar number of ripe apples from site C were also retained and inspected for pupae.

The population emerging from walnuts in January 1983 was maintained on laboratory medium for a week to insure fertility, and then given a choice of four oviposition substrates: a quartered commercial apple, a stendor dish (4.5 cm diam) of fresh medium, a quarter of a walnut husk and one-half of a native crabapple. The latter two were determined to be free of natural population larvae. Substrates were inspected daily, numbers of eggs recorded and eggs removed. Apple piece and medium were changed twice during the 6-day period; walnut husk and native crabapple were replaced every 2 days to assure moistness.

Native crabapples collected in May 1985 were inspected for *Chymomyza amoena* eggs. Those with eggs were individually placed in plastic cups over moistened potting soil (vermiculite) and capped with aluminum foil. To determine pupal location, potting soil was later transferred to a beaker of water and the number of pupae floating to the top were counted (Carson and Heed, 1983); the difference between the number of pupal cases in soil and the total number emerging from the piece of fruit represents the number pupating inside the crabapple. The same process was repeated in 1986. Experiments provide comparisons to similar work in Virginia also in 1985 and 1986.

Four pairs emerging from native crabapples in May 1985 were individually presented with two immature apples (matched for size), one of which had two *Chymomyza amoena* eggs placed on it, and a dish of medium to assure fertility. Occasionally a pest larva in an immature apple broke the surface, depositing frass. Three oviposition trials were made over 7 days. Apples were then pooled to record emergence. Pairs were also pooled and given commercial apple pieces. Numbers emerging were compared. In 1986, females emerging from native crabapples were given simultaneously a frassy unripe apple and commercial apple supplied with frass in oviposition choice tests extending over a 3-day period. Three replicates were carried out at MLBS.

5. *Behavior of* Chymomyza amoena *in Virginia.*—Work began in July 1985 to undertake more detailed comparisons of this species in the mid-South with Michigan *C.*

amoena. Apples were collected from the ground and frassy or damaged apples pulled from the trees. Two collections were made in July and one in August. Apples were inspected for *C. amoena* eggs, location was recorded and those with eggs individually placed in plastic cups over moistened potting soil; duration of emergence was recorded. Cultures were transported back to East Lansing to obtain complete records. Pupal location was determined as previously described.

Two collections were also made in the noncommercial orchard near Danville in August 1985. Their spraying program failed due to heavy rains.

Work in summer in 1986 confirmed extensive use of apples in Virginia for breeding, added more data on oviposition on fallen and unfallen fruit, and on the failure of many eggs to hatch later in summer. Apples collected in June and July which had been found to contain *Chymomyza amoena* eggs were dissected on 15 August to score for the presence of frass, if not apparent when apples were initially gathered.

Early *Chymomyza amoena* adults emerging from Virginia apples collected in 1985 were mated with Michigan *C. amoena*. The latter were the F_1s of native crabapple emergees allowed to oviposit on unripe or ripe apple. Substrate for the crosses was commercial apple supplied with frass. In 1986 reciprocal crosses were made between Michigan *C. amoena* and emergees from apples from Piedmont Virginia areas: Pamplin and Danville. Time to oviposition, and from oviposition to hatching and fertility of F_1s was determined. Again substrate was apple + frass until return to East Lansing.

6. *Other insect species.*—At all localities emergence of other species from collections yielding *Chymomyza amoena* has been recorded. This provides a geographical, seasonal and substrate record on other drosophilids and pest insects.

Comparison of initial emergence dates within Michigan and between Michigan and the mid-South from apple substrates, and emergence from individual native crabapple *Malus coronaria* and domestic apple *M. pumila* fruits to compare heterogeneity have been made by ANOVA (Sokal and Rohlf, 1981). Chi square and contingency chi squares have been computed according to Strickberger (1985), and G-statistics according to Sokal and Rohlf (1981).

RESULTS

1. *Duration of emergence.*—Emergence from ripe apples (*Malus pumila*) at East Jordan in summer, native crabapples (*M. coronaria*) in May, and immature and ripe apples in mid-Michigan in summer can extend over a month (Table 1). Emergence from late autumn/winter collections is completed more quickly whether from walnuts in 1983 and 1984 or apples (Band and Band, 1980, and here). Walnuts in 1984 were soft, spongy and more heavily used for oviposition than in previous years. Nevertheless, all 125 adults from the November-collected walnuts emerged in 10 days. Emergence from mid-South collections conforms to the Michigan pattern, although emergence from overwintered apples begins sooner. Emergence from plums in 1987 in early summer at East Lansing also began on day 22 in the laboratory but did not continue as long as from apples. Emergence from adjacent unripe fallen apples by July 1987 was as lengthy as in September 1984 from ripe apples.

Initial emergence from apples in northern lower and mid-Michigan and from the mid-South in summer is statistically comparable (Table 2). Calculations are based on collections through 1984. Initial emergence from overwintered apples in both northern lower and mid-Michigan (Band and Band, 1980, and here) is also comparable and begins earlier at 22 C than in summer. However, the earlier emergence from overwintered apples in Virginia is significant. In 1986 the one *Chymomyza amoena* emerged on day 13. In late May 1984 a *C. amoena* adult also emerged from overwintered apples collected at site B (MI) after only 8 days at 22 C.

2. *Duplication of the lengthy emergence period from apples.*—Laboratory populations grown on apples in summer 1978 yielded progeny in 36 days. Emergence from summer-collected apples begins earlier (Tables 1 and 2). Total range achieved by the F_2

TABLE 1.—Examples of emergence of *C. amoena* from different substrates in Michigan and the mid-South

Location	Date	Site	Substrate	No. emerging	Duration of emergence days
Michigan					
E. Jordan	July 1978		3 ripe apples	15	25-51
	Oct. 1978		3 ripe apples	10	22-38
	Oct. 1978		3 ripe apples	10	27-49
	Oct. 1980		12 ripe apples	62	21-50
	Aug. 1981		? ripe apples	46 +	19-52
St. Johns	March 1980		7 apples	3	14-18
	March 1981		24 apples	9	13-18
Lansing	March 1980		7 apples	2	19
Lansing	July 1981	A	18 immature apples	58 +	22-60
	Aug. 1981	A	15 ripe apples	160 +	23-64
Wmston*	May 1982		27 *M. coronaria*	48 +	17-62
Lansing	Jan. 1983	A	22 walnuts + husks	17 +	16-26
	Nov. 1983	A	25 walnuts + husks	12	21-23
E. Lansing	April 1981	RL**	8 apples	11	15-19
	Sept. 1984	B	9 ripe apples	44	24-54
	Nov. 1984	D	12 walnuts + husks	125	18-28
	June 1987	B	93 unripe plums	25	22-29
	June 1987	B	33 immature apples	20	21-32
	July 1987	B	58 immature apples	110	23-54
Mid-South					
Danville	April 1984		175 apple pieces	10	8-24
Eden	June 1984		14 immature apples	22	22-47

* = Williamston
** = Rose Lake, overwintered collection
\+ = some larvae used in supercooling work (Band & Band, 1982a,b, 1984)

TABLE 2.—Comparison of the initial emergence dates from spring-overwintered and summer-collected apples in northern lower and mid-Michigan and in the mid-South (NC). Data are based on the earliest emerging individual per collection after maintenance at 22 C spring and summer (MI) or room temperature spring and summer (NC). 95% confidence limits are given for the combined Michigan samples per season and for the mid-South in the summer

Location and season	No. collections	Initial emergence date $\bar{x} \pm$ SE	Confidence limits Lower	Confidence limits Upper
Michigan				
Spring				
E. Jordan	5	16.6 ± 1.6		
mid-Michigan	4	15.0 ± 1.7		
combined	9	15.9 ± 1.1	13.4	18.3
Summer				
E. Jordan	13	24.8 ± 1.1		
mid-Michigan	6	21.2 ± 0.9		
combined	19	23.7 ± 0.9	21.8	25.6
Mid-South				
Spring	1	8		
Summer	2	24.7 ± 1.8	17.1	32.2

of the F_1 crosses (Table 3) is comparable to the natural population data in Table 1. An initial emergence date at day 26 and a final emergence date at day 52 spans 27 days or almost a month. Emergence periods of the F_2 progeny from the F_1 fast and slow groups do not overlap; the lengthy emergence from substrates collected in late spring through early autumn represents a genetic polymorphism (Thoday, 1961) affecting developmental rate.

3. *Intrapopulation prefertile period, interpopulation, interstate and intersubstrate fertility.*—All crosses reported in Table 4 have used high protein laboratory medium. Crosses between Michigan, Missouri and North Carolina populations were fertile (Table 4). A prefertile period before females begin ovipositing, that is longer here than in *Drosophila melanogaster*, which was evident in Table 3 where flies were raised on apples, is reproduced in crosses using media-grown females and males. Results for nonaged parents do not differ when parents come from different localities, different states or the same locality collected in different years and maintained in separate populations. Oviposition is slightly but significantly faster when nonaged males and females come from the same population ($F_{1\ 27} = 12.25$; $P < 0.005$). However, oviposition begins in 3 days when adults are aged prior to setting crosses.

Crosses between emergees from different substrates are also fertile. When parents are not aged, apple/crabapple crosses have a prefertile period comparable to intrapopulation crosses whereas apple/walnut crosses resemble interpopulation crosses. This difference between type of hybrid is significant $F_{1\ 10} = 5.96$; $P < 0.05$).

TABLE 3.—Evidence of a developmental rate polymorphism in apples

F_1 emergence	Min. days to oviposition	No. transfers*	F_2 first and last emergence dates	No. emerged
36-39 days	9 days	2	26-36 days	19
42-46 days	6 days	5	28-52 days	47
47-49 days	11 days	1	38-44 days	15

* = transfer to fresh apple piece

TABLE 4.—Comparison of *C. amoena* crosses within and between states and different substrates using not-aged or aged parents. MI = Michigan; MO = Missouri; Other includes Missouri and North Carolina; EJ = different E. Jordan, MI stocks

Crosses	No.	Average prefertile period in days	Oviposition to hatching	F_1 fertile
Not aged				
MI	7	6.4±0.7	3.1±0.4	—*
Other	5	5.2±0.6	2.4±0.3	—*
MIxMI	4	9.5±1.6	2.8±1.4	—*
MIx other	9	8.7±1.0	2.7±0.5	—*
EJxEJ	4	9.0±0.6	3.5±0.3	yes
Hybrids with apples				
crabapple	4	6.5±0.5	2.8±0.5	yes
walnuts	8	9.3±0.8	3.2±0.5	yes
Aged				
MIxMI	6	3.0±0	1.7±0.4	yes
MIxMO	6	3.8±0.5	1.3±0.3	yes
MO	2	3.0±0	4.0±0	yes

* = not tested

4. *Adult behavior, oviposition, emergence and reproduction of captured pairs.*—Capture-in-the-air was observed once in an East Jordan and once in a Missouri laboratory population. The pair glide to the ground, or bottom of the population cage, where mating may occur. Assault-type mating attempts (Wheeler, 1952) are more frequently observed in this species. In laboratory culture males may approach females or females approach males. A male may tap the female before attempting to copulate.

Both sexes have the capacity to engage in sidewise movements, wing-wave, vibrate the wings, pulsate the abdomen, and to splay the front feet and "rush" an opponent. Aggression between males leads to lengthy "battles" but no real damage to combatants. Aggressive behavior can also be observed in tephritids (Dean and Chapman, 1973; Bush, 1975b) and Hawaiian *Drosophila* (Carson *et al.*, 1970; Spieth, 1982). Female-female encounters are less overtly pugilistic but are accompanied by much wing-waving and pulsating the abdomen.

Female avoidance of males in the laboratory is by uplifted abdomen with wings outstretched at 90° angles to the body. This male-mating avoidance stance by females has been described for other *Drosophila* (Ehrman and Parsons, 1981). In nature, females do not approach males until past their prefertile period. Pairs captured in 1981 and 1982 produced eggs in 3 days, as when flies are aged in the laboratory before crossing. The 1981 group was on media + apple; the 1982 pair was on apple only.

In both 1981 (Table 1) and 1982 (Table 5) a total of 35 of 43 immature fallen apples on which flies were displaying (wing-waving) already had *Chymomyza amoena* eggs. Thus there is a significant probability that a displaying adult will be on a piece of fruit on which a *C. amoena* female has oviposited ($\chi^2_2 = 15.72$; $P < 0.001$). In *Drosophila* both sexes can be attracted to sites where gravid females of their species have previously been (males: Spence *et al.*, 1984) or have oviposited (females: Mueller, 1985).

Population size in summer is small. Only 10 *Chymomyza amoena* adults were counted on fallen apples in a week of observations at East Jordan in 1979; 0 were seen in 1981 during collecting trips for apples. The maximum number of *C. amoena* counted at any one time was 25 at site A in August 1981. Adults may be visible only sporadically. Adults were seen in early July, then on 31 July 1981 at site A, in the 1st weeks of July, August and September in 1984 at site B. Four were counted each time at site B in 1984.

Seven of 263 firm fallen *Malus coronaria* fruits collected in October 1981 had eggs or larvae in plum curculio scars or other damaged areas. Three acorns with holes, of 115 collected at site D in February 1985, contained *Chymomyza amoena* eggs.

At East Jordan in northern lower Michigan females make little use of hard immature fallen apples. In mid-Michigan this is an initial summer oviposition niche. Tables 5 and 6 demonstrate that female oviposition on immature fallen apples is preferentially in scars made by earlier ovipositing females of pest species, in holes from their maturing larvae or in frass. Confirmation that females seek apples attacked by prior pests was obtained in 1985. Of the 179 immature fallen apples gathered 13 June, 31 had internal frass, of which seven contained 23 *Chymomyza amoena* eggs. *Chymomyza amoena* females later fly into trees to oviposit on frassy fruit (Table 6) as observed in 1982. On the ground they consistently seek frassy fruits for oviposition (Tables 6, 7).

In two collections in June 1987, 132 unripe fallen plums at B had 25 plums with a total of 57 *Chymomyza amoena* eggs. Numbers per fruit varied from 1 (12) to 6 (2). Plums had been attacked by plum curculio. By 14 June females were ovipositing on adjacent fallen unripe apples. At the site C farm, 94 unripe plums had only one with *C. amoena* eggs. Trees were in full sun. Collections of ripe plums had failed to produce evidence of *C. amoena* oviposition.

Table 5 also shows that females will oviposit on damaged surfaces of riper fallen apples. The 1982 data at B confirm the ability of this species to breed in unripe frassy and ripe apples, noted in Table 1.

Ten pupae were counted in frass and one in apple flesh in the three smaller unripe apples. Larvae from the riper apples from C also pupated within the substrate; 10 pu-

TABLE 5.—Distribution of *C. amoena* eggs on apples and pears in 1982

Ground or tree	Month	Site	Fruits	No.	Location					
					Calyx	Stem	Scar/hole	Split/crack	Gnawed/open	Total
Ground	June	B	im. apples	17(21)*	27	21	66		not applicable	114
Ground	July	C	ripe apples	22	8	24	36	45	37	150
Tree	August	B	pears	20(28)**	140	2	28		not applicable	170

* number in parentheses indicates the total number of small green immature fallen apples on which flies were observed
** number in parentheses indicates the number of pears collected from the tree with and without frass

TABLE 6.—Location of *C. amoena* eggs with frass in pears on tree (site B, August 1982) and small unripe apples on ground in early July 1983 (7 from site A, 11 from site B)

Location of frass	Location of *C. amoena* eggs										
	Pears on tree					Apples on ground					
	No.	Stem	Calyx	Hole	Total no. eggs	No.	Stem	Calyx	Hole	Scar	Total no. eggs
No frass	8*	0	0	0	0	2	2	1	0	0	3
Stem	2	0	0	0	0	0	0	0	0	0	0
Calyx	15	1	140	0	141	3	0	14	0	2	16
Hole	2	1	0	28	29	5	0	4	11	0	15
All 3 areas	1	0	0	0	0	0	0	0	0	0	0
Inside	0	0	0	0	0	8	3	7	11	5	26
Totals	28	2	140	28	170	18	5	26	22	7	60

*Pulled from the tree to test the hypothesis that if there were no frass there would be no *C. amoena* eggs present pae were counted in apple flesh.

Walnut emergees prefer to oviposit on softened endemic crabapples. Females laid 277 eggs on crabapple pieces, 18 on apple, eight on walnut husks and three in medium ($\chi^2_3 = 705.5$; $P < 0.001$).

Mean first emergence dates determined from individual softened overwintered *Malus coronaria* in 1985 and 1986 (Table 8) agree with earlier years from both crabapples and apples (Tables 1 and 2) as does the lengthy duration of emergence. Total numbers of eggs counted, however, can underestimate the number actually present. F-tests of emergence from the individual crabapples (a,c) in each year show significant heterogeneity; a group of flies emerging after 49 days in 1986 produced an F_1 36 days after transfer to fresh apple. Both are in agreement with a polymorphism for developmental time indicated in Table 3. Some larvae leave the substrate to pupate.

Females emerging from native crabapples laid 87 eggs on immature apples which had been "seeded" with two *Chymomyza amoena* eggs, 42 on the other apples which lacked eggs ($\chi^2_1 = 22.2$; $P < 0.005$). Of the 42, 21 were laid in frass deposited by a pest larva which broke the surface. However, equal numbers of F_1s emerged from unripe apples (52 adults) and ripe apples (51 adults) in 1985. Presented frassy unripe, immature apples and frassy ripe apple pieces in 1986, females preferred to oviposit on the former. Seventy-six adults emerged from the frassy unripe apples, 32

TABLE 7.—Distribution of *C. amoena* eggs on 18 parasitized apples at site B in July 1984. All apples gathered

No. eggs	No. apples	No. in frass	Total no. eggs
0	87	0	0
1	7	4	7
2	2	4	4
3	2	3	6
5	3	15	15
8	2	16	16
12	2	24	24
Totals	105	66	72

TABLE 8.—Comparison of emergence data of *Chymomyza amoena* from Michigan endemic crabapple fruits (*Malus coronaria*) and Virginia apples (*M. pumila*) at Mt. Lake in 1985 and 1986. Heterogeneity of emergence from individual fruits in each collection is also indicated

	1985		1986	
	M. coronaria	*M. pumila*	*M. coronaria*	*M. pumila*
No. fruits	9	5	20	5
Adults per fruit	13.6 ± 1.7	8.2 ± 2.2	8.8 ± 1.0	3.2 ± 1.1
First emergence date	25.6 ± 3.8	23.8 ± 4.7	22.1 ± 2.2	31.8 ± 1.5
Avg. emergence date	32.8 ± 0.9[a]	29.5 ± 1.7[b]	29.2 ± 0.8[c]	33.2 ± 0.9[d]
Emergence duration	42 days	40 days	44 days	25 days
No. eggs counted	31	19	88	33
No. adults emerged	122	41	176	16
% pupating in "soil"	37.9	26.9	28.0	81.0
Collecting date	5/12/85	7/16-17/85	5/30/86	6/20/86

a: $F_{8\ 113} = 16.63$; $P < 0.005$
b: $F_{4}'_{36} = 2.74$; $P < 0.05$
c: $F_{19}'_{156} = 16.23$; $P < 0.005$
d: $F_{4}'_{11} = 4.73$; $P < 0.05$

adults from the ripe apples ($\chi^2_1 = 17.0$; $P < 0.005$). The data are the pooled results of the 1986 replicates, which are similar. The oviposition data of walnut emergees on native crabapples and of crabapple emergees on immature apples agree with the interfertility of apple, crabapple and walnut emergees of Table 4.

5. *Behavior of* Chymomyza amoena *in Virginia.*—The 41 adults emerging from five apples collected in 1985 (Table 8) agree favorably with the Michigan emergence data from apples shown in Table 1 and with emergees from native crabapples in initial emergence date, significant heterogeneity of emergence dates, duration of emergence and the fact that larvae can pupate either in the substrate or soil. The lower numbers emerging from apples in 1986 may reflect a reduced number of adults because of the southern drought, reduced survival in apples because of dryness, or both (Table 8). Duration of emergence from apples gathered at Pamplin was 25 days, from apples gathered in Danville, 39 days. Apples from each locality were grouped into several containers as in initial Michigan work. Emergees from all Virginia sites were used primarily in electrophoretic work (Band and Band, 1987). Despite the larger numbers of eggs counted in 1986 (Table 9), fewer hatched than in 1985.

Females also use unfallen fruits for oviposition in Virginia (Table 9). All apples were parasitized and *Chymomyza amoena* eggs were in frass except for those on the Station grounds, which developed splits. Data from additional sites in 1986 confirm a general tendency for this species regularly to make use of fruits on the ground and on trees. Turelli *et al.* (1984) found no evidence that drosophilids in California oviposited on unfallen fruits.

Females in Virginia oviposited in association with frass or riper apples during the second half of summer 1985 (Table 10). Earlier in summer, preference is for brown rotting apples without frass or frassy unripe apples (Table 11).

In both years this species was captured on wood among other chymomyzids. In 1985 both inter- and intraspecific courting was observed; *Chymomyza amoena* is the only banded-wing species. A male in 1985 was successfully mated with Michigan females (Band, 1986a). Four adults were seen at MLBS in the 5-wk period in 1985 although five adults were counted on apples in the Danville neighborhood in June 1986.

Reciprocal crosses between Michigan and Virginia flies emerging from Rt. 700 sites in 1985 produced 31 F_1s which gave F_2s when transferred to high protein medium upon return to East Lansing. In 1986 reciprocal crosses between Michigan and Pamplin flies produced a total of 30 F_1 hybrids; reciprocal crosses between Michigan and Danville flies produced a total of 21 F_1 hybrids. Average time to oviposition was 5.3 ± 0.3 days;

TABLE 9.—*C. amoena* oviposition on apples at two locations in Virginia in summer 1985 and three locations in summer 1986 to demonstrate the use of fallen and unfallen fruits

Year	Place	Site	Location	No. apples gathered	% with eggs	Total no. eggs	No. emerged*
1985	Mt. Lake	mid-700	tree	56	5.4	16	11
			ground	111	37.8	188	37
	Danville	orchard	tree	29	7.7	4	3
			ground	35	11.4	6	1
1986	Mt. Lake	MLBS	tree	9	10.0	4	—
			ground	41	46.3	184	7
		mid-700	tree	25	8.0	3	—
			ground	100	51.0	395	36
	Blacksburg	orchard	tree	46	4.4	3	—
			ground	149	27.5	132	15

*apples in the final collections in 1986 were not held for complete emergence, but dissected to score for the presence of frass internally oviposition to hatching was 1.3 ± 0.3 days. Interstate crosses of flies from natural substrates agree well with intrastate results (Table 4). Three of the four F_1 hybrid crosses were fertile. Reciprocal crosses between Pamplin and Danville flies produced 35 F_1s which were fertile. Danville females produced low numbers of progeny in all crosses.

6. *Other insect species.*—In Michigan, *Drosophila melanogaster* and *D. simulans* emerged from winter-collected ornamental crabapples in 1983. Only the otitid *Euxesta notata* has emerged from winter-collected orchard apples, six in 1985-1986, one in 1986-1987. Adults of this species also wing-wave; larvae are more similar in morphology to *Rhagoletis*. In 1984 and again in 1986, *Grapholitha prunivora* emerged from overwintered apples in the S. This species has been found in native crabapples in winter in Michigan but not in apples collected by the author (*but see* Chapman, 1973). Two other fly species and another Lepidoptera also emerged from apples collected in Virginia in April 1984.

In summer, other drosophilids have emerged before *Chymomyza amoena*, true pests after *C. amoena*. In Michigan no other drosophilids have shared immature fallen frassy apples with *C. amoena*. Emergees from ripe apples include: *Drosophila melanogaster* and *D. algonquin*, apple maggot *R. pomonella* and codling moth *Cydia pomonella* at East Jordan; *D. melanogaster*, *D. simulans*, *D. immigrans*, *D. robusta*, *D. affinis*, *D. algonquin*, *Rhagoletis pomonella* and *Cydia pomonella* in mid-Michigan. Apples and walnuts at site A were invaded by *Drosophila* in 1985; *D. busckii* was in walnuts but not apples (Band, 1986c). *Rhagoletis suavis* larvae have been present in walnut husks but none have completed development within a single season. Plum curculio *Conotrachelus nenuphar* emerged from unripe plums in 1987.

Five other drosophilids shared unripe frassy apples in Eden in 1984; *Drosophila affinis*, *D. busckii*, *D. immigrans*, *D. melanogaster* and *Scaptomyza ajusta*. *Grapholitha prunivora* also emerged. In 1985, plum curculio weevils emerged from apples collected in mid-July at mid-700; *D. immigrans*, *D. melanogaster* and *D. affinis* emerged from late July apples along Rt. 700 as did *R. pomonella* and *G. prunivora*. No *Drosophila* emerged from apples yielding *C. amoena* in 1986 in the Blacksburg-Mt. Lake area despite the presence of *Drosophila* eggs. *Drosophila melanogaster* and *Cydia pomonella* emerged from orchard apples

TABLE 10.—Location of *C. amoena* eggs on and in apples gathered at Mt. Lake, Va., in July and August 1985

Type	Calyx	Stem	Hole/Scar	Bract	Outside	Total
Frass	19	1	7	2	0	29
Brown*	4	1	8	0	1	14
Ripe (nf)	1	1	14	5	0	21
Immature (nf)	0	0	3	0	0	3
Totals	24	3	32	7	1	67

* = small brown rotting
nf = no frass; ripe = red; immature = green

TABLE 11.—Oviposition preference of *C. amoena* among frassy and nonfrassy apples in July in western Virginia

Type of apple	Frassy	No frass	Total
Unripe rotting (brown)	6	19	25
Unripe firm (green)	36	17	53
Ripe (red)	7	3	10
Totals	49	39	88

d.f. = 2; G = 14.6; P<0.005, indicating heterogeneity among types of apples used collected in Danville in 1985, those collected in a neighborhood in 1986 yielded *D. affinis* group females and one from the *D. quinaria* group in addition to diastatids. Apples given to a relative from a noncommercial source in June 1986 were also discovered to have been parasitized and to have *Chymomyza amoena* eggs.

DISCUSSION

Chymomyza amoena is a low density, furtive species that may now be breeding and overwintering in apples throughout its range in eastern North America. This species was evidently breeding in native fruits and nuts prior to the introduction of the domestic apple. The ability to produce a new generation within a month's time assured the need for multiple hosts and mitigated against host-specific speciation as has occurred in *Rhagoletis* (Bush, 1975b).

Jefferson in 1781 listed wild crabapple, plum, black walnut and seven species of oak among the plants native to Virginia (Jefferson, 1955). Crabapples were extensively distributed throughout North America (Warder, 1867; Beach *et al.*, 1905). Barnes and Wagner (1985) include crabapples, plums, black walnut and oaks among native Michigan trees. Crabapples, plums and oaks are of world-wide distribution in the Northern Hemisphere (Wyman, 1971). *Chymomyza amoena* has been found in Czechoslovakia (Bachli and Roche-Pita, 1981, 1982). The cooccurrence at Mt. Lake of *C. amoena* and *C. caudatula*, which has a Holarctic distribution (Wheeler, 1981), provides additional support that *C. amoena* may also be in the Nearctic and Palearctic regions. In assessing the results of our study on this rarely observed species it is useful to refer back to our initial five questions.

1. How do females oviposit and larvae gain entrance into firm substrates? Reliance on other insects to attack native substrates in their firm state indicates that *Chymomyza amoena* was preadapted to invade domestic apples and other fruits. Dobzhansky (1965) defined domestic species as those associated with human habitation: in orchards, gardens and places of food processing and storage. *Chymomyza amoena* may be our "oldest domestic North American drosophilid." Mixed orchards of apple, peach, pear, plum, apricots and quince were common in colonial states by the end of the 17th century (Beach *et al.*, 1905). Weevils were attacking plums, apricots, nectarines and peaches by 1791 (Betts, 1944). Both codling moth and plum curculio larvae were identified in orchard apples in May in the mid-South by the middle of the 19th century (Fitz, 1872). Therefore, earlier and separate invasions of apples and other fruits probably occurred in the E and S prior to Michigan's established date of 1891 (Band and Band, 1982b).

Chymomyza amoena is the latest species among the drosophilids to be associated with other insects. African lissocephalids preferentially oviposit around holes made by fig weevil larvae, if available (Lachaise, 1977). *Drosophila buzzatii* and *D. aldrichi* rely on the *Cactoblastis castorum* moth to attack *Opuntia* cactus in Australia (Barker and Mulley, 1976; Barker, 1982). Frass-breeding has recently been documented for three species in Hawaii: *D. crucigera*, *D. punalua* and *D. simulans* (Heed, 1968). Drosophilids in the Cameroons in the 1930s were reported to be breeding in the frass of Lepidopterans attacking cotton (Lachaise and Tsacas, 1983). Recent interest has developed in niche-sharing (Salt, 1983; Atkinson and Shorrocks, 1984; Shorrocks *et al.*, 1984; Diamond and Case, 1987).

2. Were genetic changes involved in host expansion? Walnut emergees prefer to oviposit in native crabapples. Adults from native crabapples readily oviposit and larvae develop in apples. Unripe plums may also provide a brief breeding niche in early summer. Some tortricids are also thought to have moved easily from native fruits into introduced apple (Chapman, 1973; Roelofs and Brown, 1982). In both apples and walnuts, *Chymomyza amoena* larvae may or may not pupate in the substrate, a characteristic shared with *Scaptomyza* (Grossfield, 1978). Potential (summer) and actual (winter) larval coldhardiness polymorphisms exist in all major breeding/overwintering substrates: native and ornamental crabapples, apples and walnuts (Band and Band, 1984, 1987).

Other species in Indiana have totally switched coldhardiness type during the 1980s (Duman, 1984).

3. Does small population size promote speciation? Templeton (1980a,b, 1981, 1982) and Barton and Charlesworth (1984) have argued that founder effects do not necessarily lead to speciation. To date, there is no evidence that invasion of the apple niche or small population size has promoted speciation. The ability of females to selectively oviposit on parasitized immature fallen apples having no visible frass may promote a more ready acceptance of a male from a similar substrate. Thus the potential for speciation via host-race formation exists. However, females captured with males in nature produce fertile eggs in 3 days, as when laboratory crosses are set with flies aged a week prior to crossing. The interim period between emergence and fertility, the ephemeral nature of the endemic host plant breeding substrates and the tendency of females to oviposit where other pests or their own species have oviposited may reduce the tendency for inbreeding and for host-race formation. As yet, there is no evidence for parthenogenesis.

Behaviorally, populations remain similar over a wide geographical area. This includes capture-in-the-air mating, oviposition on unfallen fruits, elevated abdominal position in females, heterogeneity of developmental rate, larval pupation within substrate or soil, association of adults with wood and with fruits. Niche expansion into apples has not been subject to founder effects or loss of behavioral diversity. Michigan populations have been interfertile and have produced fertile progeny with *Chymomyza amoena* from other states (Band, 1981c and here). Studies between Virginia mountain and Virginia Piedmont populations still await completion; only the Pamplin population was successfully maintained past October 1986.

Chymomyza amoena appears to be the only North American drosophilid breeding in both unripe fallen and riper unfallen fruits over a wide area. Claims that *Drosophila mimica* bred in unripe *Sapindus* fruits (Barker, 1983) were mistaken in view of the findings of Richardson and Johnston (1975) that both *D. mimica* and *D. imparisetae* used rotting *Sapindus* fruit. *Drosophila* species on unfallen pears in Michigan were on overripe fruits (Band, 1986b).

4. Is developmental polymorphism recent, or does it predate apple invasion? The polymorphism for developmental rate that accounts for the lengthy emergence from Michigan apples also appears to exist among emergees from native crabapples and from apples in the mid-South. Further evidence in support of a general polymorphism is the fact that flies emerging from crabapples over days 50-53 produced an F_1 in 36 days. Multivoltinism has not given way to univoltinism as in *Chymomyza costata* in northern Europe (Hackman *et al.*, 1970; Lumme and Lakovaara, 1983). However, populations from Michigan's Upper Peninsula or from Canada have not been analyzed. Rapid and slow developers also occur in a variety of other dipterans (chironomids: Danks, 1978a; pitcher plant mosquitoes: Istock *et al.*, 1976a,b; apple maggot: Dean and Chapman, 1973; *see also* Parsons, 1983) and in *Drosophila melanogaster* (Cavener and Clegg, 1981; Marinkovic *et al.*, 1987).

5. Are there geographical differences between populations? Conservation of behavioral diversity over a wide geographical area, including developmental polymorphism, contrasts with some behavioral differences that are observed within Michigan, and between Michigan and Virginia flies. No oviposition on unfallen fruits was found at East Jordan, Mich., where climate can support about one and one-half generations between July and mid-September. The significantly more rapid emergence from overwintered apples in Virginia suggests that either the pupal stage is entered earlier in spring or else the pupal stage may overwinter in the mid-South (larvae overwinter in the N). The fact that adults emerge within 15 days when overwintering substrates are kept at 22 C or larvae acclimated to subzero temperatures are returned to 22 C (Band, 1981a,b and here; Band and Band, 1980) supports Tauber *et al.* (1982) that cold will maintain diapause in temperate species. These latitudinal differences are possibly sharp enough

to affect overwintering but not sharp enough to cause a transition to univoltinism as in Northern Europe (Lumme and Lakovaara, 1983). Other differences emerge in niche sharing. Differences may be real with respect to overwintering; more species in the mid-South are successfully able to overwinter also in orchard apples. Females in Virginia also tend to make greater use of unripe rotting apples for oviposition than in Michigan.

Although adaptation has become controversial (Gould and Lewontin, 1979; Krimbas, 1984, *but see* Mayr, 1983; Wallace, 1984), it is clear that species overwintering in an immature stage in exposed substrates are subject to strong selection. This has led to geographical differences between populations of the same species (Danks, 1978b, Baust, 1981; Baust and Lee, 1981). Overwintering differences between Michigan and mid-South *Chymomyza amoena* are not unexpected. Summer and winter populations of the same species may also be physiologically and biochemically different (reviews: Duman and Horvath, 1983; Baust and Rojas, 1985; Zachariassen, 1985). Winter dormancy is not a static phase of the life cycle. *Chymomyza amoena* larvae have been found to respond dynamically to seasonal changes (Band and Band, 1982a,b, 1984, 1987), analogous to *Drosophila auraria* adults in Japan (Higuchi and Kimura, 1985), and to accumulate proline as do other Diptera, Lepidoptera, Coleoptera and plants under stress.

Recently, the relation of the chymomyzids to the *Drosophila* has become controversial (Beverley and Wilson, 1984; MacIntyre and Collier, 1986). MacIntyre and Collier (1986), on the basis of work with α-GPGH, included the chymomyzids in the *Drosophila*. Beverley and Wilson (1984), on the basis of work with LSP-2, argued that the chymomyzids diverged early from the other drosophilids. Observed behaviors of wing-waving, capture-in-the-air and assault-type mating are both non-*Drosophila* and nondrosophilid, while the accumulation of proline in winter argues for a biochemical conservatism that transcends insects (Band and Band, 1987).

The present work on *Chymomyza amoena* reaffirms the need of drosophilids for damaged or rotting substrates for oviposition and indicates that interspecies relationships to other organisms are readily formed (Carson and Heed, 1983). It also indicates that behavior incorporated into phylogenetic studies may provide a more accurate assessment of chymomyzids in relation to other drosophilids and other Diptera. Rightly regarded as a separate genus, chymomyzids may be important in showing that microevolution adequately accounts for macroevolution (Dobzhansky, 1941; Patterson and Stone, 1952; Carson *et al.*, 1970; Wright, 1982a,b).

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