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EVOLUTION OF FOOD PREFERENCES IN FUNGUS-FEEDING *DROSOPHILA*: AN ECOLOGICAL STUDY

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Host plant selection by insects has evolved through various interactions with their environments. In phytophagous insects, the chemistry and ecology of plants are important factors governing host selection. Plants have unique chemicals, some of which, such as toxicants, have evolved as defense mechanisms against insect attacks, and plants also show species specific distribution and phenology. Insects, then, have evolved metabolic systems to utilize such chemicals or to detoxify, and also bionomic strategies to adapt to the plant phenology. Specialization of phytophagous Lepidoptera is governed by interactions with host-plant chemistry, but also with host abundance and predictability (cf. Gilbert, 1979). In addition to plant chemistry and ecology, the existence of species having similar nutritional requirements influences their selection of hosts through interspecific competition. The validity of these conceptions needs to be examined in various phytophagous insects and also in insects feeding on materials other than plants. This paper deals with food preferences and nutritional requirements of the fungus-feeding *Drosophila* with reference to factors governing their selection of hosts.

The original feeding habit of the family Drosophilidae was saprophagous: fermenting or decayed plant parts such as fruits, tree sap, leaves, stems, flowers, or mushrooms. Today many species belonging to various taxonomic units continue with this original feeding habit (Carson, 1971; Kimura et al., 1977). However, several species have come to feed and breed on living or fresh plant parts, e.g., the leaf miners of *Scaptomyza* (Frost, 1924) and the flower feeders of *Drosophila* (Brncic, 1966; Pipkin et al., 1966). Kimura (1976) observed that adults of some species of *Hirtodrosophila* were attracted to fresh mushrooms. In this study, the food preferences of these fungus feeders were examined in laboratory tests and field observations in relation to the phenology and abundance of mushrooms. The species studied included three members of the *Hirtodrosophila* radiation, *D. trivittata* Strobl, *D. sexvittata* Okada, and *D. alboralis* Momma and Takada, and five species of the *immigrans* radiation, *D. immigrans* Sturtevant, *D. nigromaculata* Kikkawa and Peng, *D. brachynephros* Okada, *D. testacea* van Roser, and *D. confusa* Staeger. All species except *D. immigrans* and *D. nigromaculata* depend on mushrooms. *Drosophila immigrans* feeds on fruit and *D. nigromaculata* feeds on leaves (Kimura et al., 1977).

METHODS

Laboratory Tests

Preferences of adult flies for fresh and decayed mushrooms.—About 30 females were introduced into a container (1,300 ml) which contained fresh, intermediate, and decayed *Pleurotus cornucopiae* (Pers.) (5 g of each). Individuals of all species used in the test, except *D. alboralis,* were collected in fields in Sapporo and maintained for several days on yeast-agar medium. The individuals of *D. alboralis* were the first generation offspring of field-collected females. They were bred on *P. cornucopiae*. The decayed mushroom was obtained by leaving a fresh one for four days at 23 C, and the intermediate, for two days. The container was kept under a weak light condition at 20 ± 1 C. The number of adults attracted to these mushrooms was counted 12 times at 1-h inter-

TABLE 1. *Mean numbers (± standard deviation) of adults attracted to (A) and numbers of eggs oviposited on (O) the fresh (F), intermediate (I), and decayed (D) mushrooms, and the adult preference in feeding analysed by* t-*test (5%).*

		Fresh	Intermediate	Decayed	Preference
D. immigrans	#1 (A)	1.83 ± 1.11	4.92 ± 0.90	5.17 ± 1.34	D = I > F
	(O)	154	297	573	
	#2 (A)	2.67 ± 1.72	2.75 ± 1.82	8.00 ± 3.07	D > I = F
	(O)	311	186	399	
D. nigromaculata	#1 (A)	1.92 ± 1.31	5.42 ± 1.56	5.67 ± 1.87	D = I > F
	(O)	54	81	116	
	#2 (A)	5.25 ± 1.29	2.17 ± 1.47	6.75 ± 2.18	D = F > I
	(O)	33	18	65	
D. brachyneophros	#1 (A)	4.58 ± 2.31	3.42 ± 2.11	5.25 ± 1.60	D = F, F = I, D > I
	(O)	39	40	74	
	#2 (A)	2.33 ± 0.89	3.92 ± 1.38	4.92 ± 1.31	D > I > F
	(O)	7	17	48	
D. testacea	#1 (A)	1.42 ± 0.67	1.08 ± 1.16	2.08 ± 1.51	D > F > I
	(O)	11	32	114	
	#2 (A)	1.92 ± 1.09	2.75 ± 1.06	3.75 ± 0.62	D > I > F
	(O)	12	22	47	
D. confusa	#1 (A)	3.75 ± 1.36	2.17 ± 1.03	4.08 ± 1.78	D = F > I
	(O)	41	34	193	
	#2 (A)	4.17 ± 1.47	3.67 ± 0.98	3.33 ± 1.07	F > I = D
	(O)	95	256	376	
D. alboralis	#1 (A)	3.25 ± 0.87	2.50 ± 1.00	2.42 ± 1.44	F > I = D
	(O)	19	14	19	
	#2 (A)	2.08 ± 1.16	2.67 ± 0.89	2.00 ± 1.21	I > F = D
	(O)	13	7	12	
D. sexvittata	#1 (A)	4.75 ± 2.49	1.42 ± 1.51	1.33 ± 0.98	F > I = D
	(O)	168	152	76	
	#2 (A)	4.00 ± 2.49	0.33 ± 0.49	0.75 ± 0.75	F > D > I
	(O)	25	15	11	
D. trivittata	#1 (A)	4.75 ± 1.48	2.42 ± 1.31	0.50 ± 0.67	F > I > D
	(O)	64	73	—	
	#2 (A)	9.08 ± 3.18	3.50 ± 1.38	0.67 ± 0.65	F > I > D
	(O)	91	25	—	

vals from the introduction of the flies, and the number of eggs oviposited on them was counted after 24 h. At the same time, the parts of the mushrooms on which the eggs were oviposited were examined: stipe, upper surface of pileus, or lamella. The test was made twice.

Survivorship and egg productivity on different kinds of food.—Ten field-collected females were maintained on six kinds of food at 23 ± 1 C: an agar medium (agar 1.4%), a yeast medium (dry yeast 8%, agar 1.4%), banana, fresh *P. cornucopiae,* decayed *P. cornucopiae,* and fresh *Agaricus bisporus* (Lange). The number of dead flies and the number of eggs oviposited were counted daily for ten days, and the foods were also renewed daily.

Preferences of larvae for fresh and decayed mushrooms.—Twenty to forty larvae of the second or third instar which were cultured on the yeast-agar medium or *P. cornucopiae* were introduced into a petri dish which contained fresh, intermediate, and decayed *P. cornucopiae* (0.5 g of each), and after 3 h the number of larvae attracted to each condition was counted.

Field Observation

Seasonal changes of host mushrooms.—The phenology of mushrooms and the

TABLE 2. *Proportions of eggs oviposited on the stipe, upper surface of the pileus, and the lamella of* Pleurotus cornucopiae. *The data summed the results on the fresh, intermediate, and decayed mushrooms.*

	Stipe	Pileus	Lamella
D. immigrans	42.3	54.2	2.3
D. nigromaculata	23.2	69.7	7.1
D. brachynephros	22.0	77.4	0.6
D. testacea	29.9	68.8	1.3
D. confusa	53.9	40.7	5.4
D. alboralis	21.4	77.4	1.2
D. sexvittata	1.1	37.1	61.8
D. trivittata	—	2.4	97.6

breeding of *Drosophila* species were studied on the basis of data obtained from 1972–1977 in Sapporo (cf. Kimura et al., 1977).

Fungus preferences of adult flies.—Periodic collections of adult flies from naturally occurring mushrooms were carried out at the Nopporo Natural Forest and the Moiwa Natural Forest near Sapporo in 1975, and at the Nakagawa Experimental Forest at northern Hokkaido in 1973. The collections were made for two days at each collecting period in the beginning and middle of each month from June to October in Nopporo and Moiwa, and only at the middle of each month in Nakagawa. The collections were performed along paths or streams, along a 900 m route in Moiwa, 1,100 m in Nopporo, and 3,000 m in Nakagawa. The flies were collected from all the mushrooms which were found along the route three times a day at 13:00, 15:00, and 17:00 h.

RESULTS

Preferences of adult flies for fresh and decayed mushrooms.—Table 1 gives the mean numbers of flies attracted to and the numbers of eggs oviposited on the three conditions of mushrooms. Between the two replicates, some differences were observed as to their preferences, rates of adults attracted, or numbers of eggs oviposited. This was assumed to be due partly to differences in the physiological conditions of flies used in the test, e.g., age, period of laboratory culture, and also to the difference in mushrooms in the degree of decay. The decay did not always proceed in the same way, though mushrooms were kept under the same conditions. However, in spite of such differences, many species showed rather similar preferences in the two replicates. Four species of the *immigrans* radition, *D. immigrans, D. nigromaculata, D. brachynephros,* and *D. testacea,* preferred the decayed mushroom in feeding and ovipositing, although adult flies were sometimes attracted to the fresh mushroom. *Drosophila confusa* did not show a clear preference in feeding, but in ovipositing this species clearly preferred the decayed mushroom, while *D. alboralis* did not show a clear preference in either feeding or ovipositing. In contrast, *D. sexvittata* and *D. trivittata* preferred the fresh mushroom in feeding and ovipositing. *Drosophila sexvittata* oviposited on the decayed mushroom to some extent, but *D. trivittata* avoided the decayed mushroom for oviposition.

Ovipositing habits.—Species varied in the proportions of eggs oviposited on different parts of mushrooms (Table 2). *Drosophila trivittata* oviposited mainly on the lamellae and *D. sexvittata* on the lamellae and the upper surface of the cap. *Drosophila nigromaculata, D. brachynephros, D. testacea,* and *D. alboralis* oviposited mainly on the cap, but *D. immigrans* and *D. confusa* oviposited about equally on the cap and stipe. Although these species sometimes showed different ovipositing habits for the different conditions of mushrooms, no stable trend was observed. On a different mushroom, *Agaricus bisporus,* all the species except for *D. trivittata,* which did not oviposit on this mushroom (cf. Table 4), oviposited almost exclusively on the stipe when the mushroom was fresh.

Survivorship and egg productivity on different kinds of food.—Table 3 shows the mean survival periods (days) of adult females when maintained for ten days on the six kinds of food. On the yeast medium, banana and the fresh *Pleurotus,* all species survived very well, but on the agar medium, the fresh *Agaricus,* and the decayed *Pleurotus,* they did not. In partic-

TABLE 3. *Mean (± standard deviation) survival periods (days) of adult females when maintained for ten days on six kinds of food.*

	Agar	Yeast	Banana	Fresh *Pleurotus*	Decayed *Pleurotus*	Fresh *Agaricus*
D. immigrans	*2.9 ± 0.7	10.0	10.0	7.5	*1.0 ± 1.1	*2.9 ± 0.5
D. nigromaculata	*5.9 ± 1.1	10.0	9.2	8.5	*2.1 ± 0.5	*5.1 ± 1.6
D. brachynephros	*5.0 ± 1.5	10.0	8.9	9.3	*6.9 ± 1.9	6.2
D. testacea	6.9	10.0	9.4	10.0	7.7	7.9
D. confusa	*4.9 ± 1.5	10.0	8.7	9.5	7.9	5.5
D. sexvittata	*2.1 ± 1.1	10.0	4.6	9.1	*2.9 ± 2.3	6.0
D. trivittata	*2.6 ± 0.3	10.0	8.2	9.3	*1.5 ± 0.0	*2.1 ± 1.5

* All individuals were dead within ten days. In the remaining cases, the real survival periods were longer than the figures presented, and then the standard deviation values calculated from this test were useless.

ular, *D. immigrans* and *D. nigromaculata* survived only for a short period of time on the decayed *Pleurotus*. The agar medium and the fresh *Agaricus* were malnutritional for *Drosophila* species, but the decayed *Pleurotus* evidently was nutritional at least for some species. On the decayed *Pleurotus,* the flies seemed to die early because they were poisoned by muscoid from the mushrooms and also by toxic gases produced in the process of decay.

Table 4 shows the numbers of eggs oviposited on the six kinds of food during the survival period and the daily mean production of eggs per female during the first five days and the next five days. Because the individuals used in the test were maintained for several days on the yeast medium after collections, the egg production immediately after the start of the test was affected by the nutritional condition of flies during the maintenance on the yeast medium. Therefore, egg production during the second five days should reflect the nutritional value of the foods for the flies. The t-test ($P = .05$) was applied to analyze the differences in egg production during the second five days. *Drosophila immigrans* oviposited significantly more when cultured on banana than on yeast medium, while *D. nigromaculata* oviposited more on the yeast medium than banana. On other foods, their egg production was significantly lower. *Drosophila brachynephros* and *D. testacea* produced significantly more eggs on decayed *Pleurotus* and the yeast medium than other foods, and the latter produced significantly more on decayed *Pleurotus* than the yeast medium. Egg production of *D. confusa* was high on the yeast medium, decayed *Pleurotus,* and banana, but the differences between these foods and other foods were sometimes insignificant. *Drosophila sexvittata* and *D. trivittata* continued egg production only on fresh *Pleurotus*. On other foods, they did not oviposit, or stopped oviposition soon after the start of culture. *Drosophila trivittata* concentrated more on fresh *Pleurotus* than did *D. sexvittata*.

The rate of egg production observed in the previous test (Table 1) was sometimes inconsistent with this result. For example, *D. testacea* produced a large number of eggs on the decayed mushroom in this test, but not on the decayed mushroom in the former tests. *Drosophila immigrans* showed an opposite trend. This inconsistency was considered to be partly due to the effect of culture on the yeast medium before tests; the yeast medium was adequate for *D. immigrans,* but not for *D. testacea.*

Preferences of larvae for fresh and decayed mushrooms.—Larvae of *D. sexvittata* and *D. trivittata* were attracted about equally to the intermediate and decayed mushrooms, but those of the other species were significantly more attracted to the decayed one (t-test, $P = .05$) (Table 5).

Phenology and abundance of mushrooms in Hokkaido and seasonal changes of host mushrooms.—Table 6 shows the phenology of mushrooms in Sapporo on

TABLE 4. *Numbers of eggs oviposited by ten females on six kinds of food during the survival periods (a), and daily mean production of eggs per female during the first five days from the start of test (b) and during the second five days (c).*

	Agar			Yeast			Banana			Fresh *Pleurotus*			Decayed *Pleurotus*			Fresh *Agaricus*		
	a	b	c	a	b	c	a	b	c	a	b	c	a	b	c	a	b	c
D. immigrans	14	0.3	—	817	4.0	12.4	1,687	13.7	20.0	324	4.6	3.2	83	8.3	—	1	0.0	—
D. nigromaculata	168	3.0	1.8	1,171	12.1	11.4	723	8.4	7.3	439	7.8	0.6	161	6.4	—	171	3.7	0.0
D. brachynephros	51	1.0	0.0	367	4.3	3.0	143	2.0	1.1	184	2.9	0.9	526	8.0	5.9	8	0.2	0.0
D. testacea	109	2.2	0.2	726	8.4	6.2	288	4.8	1.3	326	4.4	2.2	1,390	16.1	20.7	120	2.4	0.3
D. confusa	189	4.0	0.0	522	4.3	6.1	525	7.1	4.7	375	5.6	2.2	483	6.4	5.3	147	2.9	1.3
D. sexvittata	150	5.3	—	243	4.1	0.7	68	1.8	0.0	490	5.7	5.2	57	2.0	0.0	76	1.6	0.0
D. trivittata	35	0.7	—	26	0.5	0.0	2	0.0	0.0	363	3.1	5.1	—	—	—	—	—	—

TABLE 5. *Proportions of larvae attracted to the fresh, intermediate, and decayed* Pleurotus cornucopiae.

	Fresh	Inter-mediate	De-cayed	No. of larvae
D. immigrans	14.6	34.1	51.2	41
D. nigromaculata	4.2	21.1	74.7	71
D. brachynephros	4.4	15.6	80.0	45
D. testacea	8.3	26.3	65.4	133
D. confusa	11.4	30.0	58.6	70
D. alboralis	5.6	11.1	83.3	17
D. sexvittata	4.4	50.0	45.7	46
D. trivittata	11.1	40.7	48.2	81

which drosophilid flies depended strongly. Five species of mushrooms—*Pleurotus ostreatus* (Fr.), *P. cornucopiae, Pluteus cervinus* (Secr.), *Coprinus micaceus* (Fr.), and *Polyporellus squamosus* (Fr.)—were observed continuously from May to September. Among these five species, the two of *Pleurotus* were found abundantly in Hokkaido, and were very attractive to the flies as indicated by the results of adult collections in Nopporo and Moiwa (Table 7) and in the Botanical Garden (Kimura, 1976). The other three species were observed frequently in the Botanical Garden, but were less abundant in the other localities. The remaining mushrooms were restricted seasonally, and their occurrence was also limited in some environments.

Breeding in the field was observed from May to September for all species. This period is adequate for two or three generations (Watabe and Beppu, 1977; Kimura et al., 1978, unpubl.). *Drosophila trivittata* used *P. ostreatus* throughout the seasons, and it occasionally used some other mushrooms of the family Tricholomataceae. On the other hand, *D. sexvittata* and *D. alboralis* used a wide variety of mushrooms, but seldom the mushrooms of *Pleurotus*. I have reared 910 individuals of *D. trivittata* from the naturally occurring mushrooms of *Pleurotus*, but only 32 of *D. sexvittata* and 12 of *D. alboralis*. *Drosophila brachynephros, D. testacea* and *D. confusa* were observed breeding on various mushrooms including *Pleurotus*. These species changed the host mushrooms seasonally, with the

TABLE 6. *Seasonal occurrences of main host mushrooms for breeding in Sapporo.*

	May 1–15	May 16–31	June 1–15	June 16–30	July 1–15	July 16–31	Aug. 1–15	Aug. 16–31	Sept. 1–15	Sept. 16–30
Pleurotus ostreatus	+	+	+	+	+	+	+	+	+	+
Pl. cornucopiae		+	+		+	+	+	+		+
Armillariella mellea		+	+	+	+			+	+	+
Oudemansiella mucida			+	+				+	+	
Ou. radicata					+	+				
Tricholomopsis platyphylla		+	+							+
Colybia erythropus			+	+						
Pholiota squarrosa				+	+		+			
Crepidotus mollis			+	+		+	+			
Pluteus cervinus		+	+	+	+	+	+	+	+	
Coprinus micaceus	+	+	+	+	+	+	+	+	+	+
Co. atramentarius			+	+	+	+			+	+
Psathyrella candolleana			+	+	+	+	+		+	
Polyporellus squamosus	+	+	+	+	+	+	+	+	+	+

phenology of mushrooms, but no particular relationship was observed between seasons and host mushrooms.

Fungus preferences of adult flies.—Table 7 gives the numbers of adults of each species collected and the percentages of adults collected from main fungus families, Tricholomataceae, Russulaceae, Amanitaceae, and Boletaceae, and also from the genus *Pleurotus*. *Drosophila unispina* Okada is a closely related species of *D. brachynephros*. In Nopporo and Moiwa, mushrooms of Tricholomataceae (*Pleurotus ostreatus, P. cornucopiae, Colybia* sp., *Leucopaxillus giganteus, Oudemansiella mucida, Tricholomopsis decola*) were observed frequently, and in Nakagawa, mushrooms of Russulaceae (*Russula* sp., *R. cyanoxantha, Lactarius piperatus*), Amanitaceae (*Amanita longistriata, A. phalloides*), and Boletaceae (*Laccinum scabrum, Tylopilus plumbeoviolaceus*) were frequent. *Drosophila trivittata* was collected considerably from the family Tricholomataceae, and particularly from the genus *Pleurotus*. In Nakagawa, only a few individuals of *D. trivittata* were collected in this study, but a considerable number was collected from *Pleurotus cornucopiae* in another survey (Kimura and Toda, 1976). On the other hand, *D. sexvittata* was attracted to various mushrooms, but few to the family Boletaceae in Nakagawa. *Drosophila alboralis* seemed to be less dependent on Tricholomataceae than the above two species. The members

TABLE 7. *Percentages of flies of each species collected from the main fungus families (Tricholomataceae, Russulaceae, Amanitaceae, Boletaceae) and from the genus* Pleurotus *in the Nopporo Natural Forest, the Moiwa Natural Forest, and the Nakagawa Experimental Forest.*

	Nopporo			Moiwa			Nakagawa			
	Trich.	*Pleu.*	Total number of flies	Trich.	*Pleu.*	Total number of flies	Russ.	Aman.	Bole.	Total number of flies
D. trivittata	62.4	53.2	109	99.8	93.0	1,784	—	33.3	—	3
D. sexvittata	66.1	8.3	672	87.9	55.7	481	59.2	32.7	1.6	444
D. alboralis	24.1	6.9	29	39.2	3.9	102	26.7	6.7	26.7	15
D. confusa	95.0	5.0	20	41.7	33.3	12	29.0	1.6	68.6	245
D. testacea	71.4	59.2	49	98.7	98.1	155	11.2	4.5	82.3	1,842
D. brachynephros	90.0	30.0	30	0.0	0.0	4	15.8	31.6	47.5	19
D. unispina	78.6	35.8	14	66.7	33.3	6	21.5	36.6	40.9	93
Others	46.9	15.6	32	25.8	21.5	93	10.7	5.3	78.7	75
Total	65.5	17.3	955	92.2	80.1	2,637	21.1	10.0	65.9	2,736

of the *immigrans* radiation were collected abundantly in Nakagawa and were attracted to various mushrooms.

Discussion

The members of the *immigrans* radiation are saprophagous, but the most suitable foods for their egg production were different: *D. testacea, D. brachynephros* and *D. confusa* for decayed mushrooms, *D. immigrans* for banana, and *D. nigromaculata* for the yeast medium. In fields, they also use different types of resources as breeding sites: *D. testacea, D. brachynephros,* and *D. confusa* depend on mushrooms, *D. immigrans* on fruit, and *D. nigromaculata* on leaves (Kimura et al., 1977). Within each type of resources, each species uses a wide variety of plant species (Dobzhansky and Pavan, 1950; Lachaise, 1974; Kimura et al., 1977). The fungus-feeders also showed a wide preference for mushrooms and have a rather similar preference to each other as observed in the result of adult collections and also in studies of Kimura (1976) and Kimura et al. (1977). Jaenike (1978) attributed their polyphagy to the unpredictability of occurrences of mushrooms. If this is true, all fungus-feeders must be polyphagous. However, species feeding on fresh mushrooms showed a host specialization in Hokkaido. Therefore, unpredictability of resource is not considered to act as an effective pressure for polyphagy, at least in Hokkaido.

The saprophagous species seem to feed on microorganisms and/or fermenting products in the main. Even the fungus-feeders produced eggs more on the yeast medium than fresh mushrooms, and further, their larvae grew up to adults on the yeast medium which contained no trace of mushroom, although the viability of *D. testacea* and *D. confusa* was very low (1–10%) (pers. observ.). Da Cunha et al. (1951) and Dobzhansky and Da Cunha (1955) suggested that the saprophagous fruit-feeders seemed to be attracted to fermenting products rather than microorganisms or substrates: pure cultures of yeasts or autoclaved banana without yeast attracted few or no *Drosophila,* but banana inoculated with yeasts was a potent attractant. Therefore, the toxic and nutritional characteristics of the substrates would be less influential in their host selection, and further chemical uniqueness of substrates may decrease by the action of microorganisms. Such a situation would support the idea of polyphagy for substrates.

However, when substrates differ considerably in chemistry, host specialization is possible, because of chemical difference of substrates themselves or differences of microorganisms or fermenting products which are due to the difference of substrates. The specialization of saprophagous species to one type of resource, i.e., mushrooms, fruit, or leaves, would be supported by such a situation. Furthermore, the exceptional monophagy was observed on those which depend on plants containing very unique or highly concentrated chemicals; e.g., *D. pachea* (Heed and Kircher, 1965) and *D. quinaria* (Jaenike, 1978). The uniqueness or high concentrations of chemicals, although their efficiency decreases in saprophagous species, may be sufficient to govern their host selection. But, many Hawaiian *Drosophila* have specialized in the decayed leaves of *Cheirodendron* in which no biochemical peculiarity is known (Kircher and Heed, 1970).

The members of the *Hirtodrosophila* radiation have left the original saprophagous habit, and they showed a preference for fresh mushrooms. This preference was observed only among adults. They take nutrients from fresh mushrooms and oviposit on them. As the individuals of *D. trivittata* and *D. sexvittata* show feeding behavior on the lamellae (Kimura et al., 1977), they may feed on spores. By microscopic observations, mushroom spores were found in their crops, but it is not certain whether the spores are their main food. On the other hand, their larvae showed a saprophagous habit, although they did not show so strong a preference for the decayed mushrooms as larvae of the members of the *immigrans* radiation.

Wiklund (1975) observed that adult and larval host plant preferences are determined by separate gene complexes in *Papilio machaon* L. and the larval preference is wider than the adult preference. He explained the relation between adult and larval host plant range by the larval mortality due to inability to accept the host plant as food. However, in *D. sexvittata* and *D. trivittata,* the separation between adult and larval preferences would have developed in a different way. Although *D. sexvittata* and *D. trivittata* have oviposited eggs on fresh mushrooms, the mushrooms decay during the period of larval growth. Then, the preference for fresh mushrooms would not develop in larvae. Similarly in saprophagous species of *Drosophila,* differences in adult and larval preferences for yeasts and bacteria were sometimes observed (Carson et al. 1956; Cooper, 1960). This difference might reflect the difference in microbe flora between the time of adult feeding and larval feeding.

The advantages obtained by feeding on fresh mushrooms are not clear. The predictability and abundance of fresh mushrooms are undoubtedly higher than the decayed ones, because mushrooms sometimes dry up without decay. However, this advantage decreases, because some mushrooms, e.g., *Agaricus bisporus,* are unsuitable for food when fresh. The intraspecific and interspecific competition may play a role in the development of preference for fresh mushrooms. Gilpin (1974) showed that larvae oviposited earlier have a competitive advantage over those oviposited later in intraspecific competition of *D. melanogaster.* If such competition occurs in nature, individuals which oviposit at the fresh stage of materials have an advantage. Once such development is initiated, it would be reinforced by competition with other saprophagous species. This idea, however, does not explain the change in adult feeding habits. Competition for foods between adult flies may work as a selective pressure bringing a change in adult preference.

In the species studied here, *D. trivittata* showed the most specialized fungus preference. Their breeding was almost restricted to Tricholomataceae (Kimura et al., 1977). *Drosophila sexvittata* also prefers Tricholomataceae, although it feeds and breeds on various kinds of mushrooms; Tricholomataceae produced 85% of total individuals of *D. sexvittata* reared in an earlier study (Kimura et al., 1977). In phytophagous insects, specialization defined at the level of host-plant families is governed by interactions with host-plant chemistry rather than by competitive interactions or relative abundance of host families (cf. Gilbert, 1979). This explanation may apply to the specialization of *D. trivittata* on Tricholomataceae. However, because chemical components of mushrooms are not well known, a further study is needed. The nutritional value of mushrooms is one of the factors determining the food preference of these species. *Agaricus bisporus* was malnutritional for adult flies when fresh. Savile (1973) assumed that heavily pigmented spores of coprophilous fungi are indigestible for herbivores because of their impermeability to many fluids. The black spores of *Agaricus* may have the same role. Then, the species feeding on spores would not show a preference for such mushrooms. In fact, the mushrooms having such heavily pigmented spores, e.g., *Coprinus* or *Agaricus,* seldom attracted *D. sexvittata* and *D. trivittata* in the field (Kimura, 1976). Plants frequently have developed defense mechanisms against herbivores, such as toxic chemicals or thorns. The pigmentation of spores may also have evolved as a defense mechanism.

Within Tricholomataceae, *D. trivittata* has a strong preference for *Pleurotus* as observed in this study and in a previous study (Kimura, 1976). This specialization would be supported by the seasonal and spatial ubiquity of these mushrooms. However, although these mushrooms are abundant, their occurrence is not so highly predictable. The life of a mushroom body is usually short and the resource is temporally patchy. Furthermore, the crops of mushrooms fluctuate yearly and seasonally in response to climatic conditions, especially rainfall. Such unpredictability of occurrence may prevent the complete specialization of *D. trivittata* on *Pleurotus*. The resource predictability and abundance also seem to govern the host specialization of tropical flower-feeding *Drosophila*. The monophagous species specialize on plants with long flowering periods and sufficient density to support *Drosophila* populations (Pipkin et al., 1966). Further, in the host specialization of *Heliconius* butterflies within Passifloraceae plants, Gilbert (1979) argued that predictability of host plant plays an important role.

Despite suitability of the *Pleurotus* mushrooms as a resource, *D. sexvittata* and *D. alboralis* did not show so clear a preference for them as did *D. trivittata,* and they seldom use them as a breeding site. They may tend to avoid these mushrooms to minimize the interspecific competition with *D. trivittata*. However, adults of *D. sexvittata* were frequently attracted to the *Pleurotus* mushrooms in the fields and they can take nutrients for survival and egg production from fresh *P. cornucopiae* under laboratory conditions.

On *Pleurotus cornucopiae,* most species oviposited eggs into the stipe and upper surface of the pileus, but *D. sexvittata* and *D. trivittata* deposited eggs into the lamella. However, the ovipositing sites vary according to the kind of mushrooms. On fresh *Agaricus bisporus,* all species deposited into the stipe. Shorrocks and Wood (1972) also observed that *D. pharelata* Meigen and *D. busckii* Coquillett oviposited eggs mostly into the stipe of this mushroom when fresh. The unique ovipositing habit of *D. sexvittata* and *D. trivittata* observed on *P. cornucopiae* may have evolved in relation to the development of a preference for fresh mushrooms and also as a result of specialization on mushrooms of Tricholomataceae. These two species are also unusual in having eggs without filaments (Okada, 1956). In Drosophilidae, species which oviposit eggs on fresh or living materials often have eggs with modified filaments or none at all, e.g., *Scaptomyza graminum* (Fallén) (leaf-miner; Okada, 1968), some of the flower feeding species of *Drosophila* (Patterson and Stone, 1952; Pipkin et al., 1966), and some of *Lissocephala* (Lachaise, 1977).

SUMMARY

The fungus-feeding *Drosophila* species of the *immigrans* radiation and the *Hirtodrosophila* radiation showed different adaptations to mushrooms. The members of the *immigrans* radiation retain the original saprophagous habit and do not specialize in their fungus preferences. They seem to select hosts not by chemical uniqueness or nutritional value of mushrooms themselves, but by microorganisms growing on the mushrooms or their products. Such a situation is considered to support their polyphagy for mushrooms. On the other hand, adults of the members of the *Hirtodrosophila* radiation have a preference for fresh mushrooms, although their larvae prefer the decayed ones like those of the species of the *immigrans* radiation. This difference between adult and larval preferences may relate to different conditions of mushrooms between the time of oviposition and the larval stages. The species which showed a stronger preference for fresh mushrooms specialized more on the family Tricholomataceae. *Drosophila trivittata* shows a strong preference for mushrooms of the genus *Pleurotus* which are found through the breeding seasons of flies in various environments in considerable abundance, but *D. sexvittata* and *D. alboralis* did not show such clear preference for these mushrooms. Predictability and abundance of mushrooms and competitive interactions between flies probably govern host specialization. Accompanied by the change in the fungus preference, *D. sexvittata* and *D. trivittata* innovated a new ovipositing habit, on the lamellae rather than the stipe or upper surface of the pileus.

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