

Diadromus pulchellus is 0.273, and the mean heterozygosity ($= H_a$ of Pamilo and Crozier⁸) can be calculated to range between 0.070 and 0.112 depending on whether Ldh-1 and Acp-2 are included in the analysis or not^{15,16}. The other 6 species reported by Menken¹⁷ have P ranges from 0 to 0.158 and H_a ranges from 0 to 0.056. The lack of variation in *Mesochorus vittator* is certainly due to the small sample size (2 males and 1 female)¹⁷ and thus cannot be compared with the congeneric species *M. nigripes* in our study. Since genetic diversity is known to have a much greater value of standard error, Graur⁶ used at least 15 loci to calculate expected heterozygosity ($= H_a$) in 188 species of insects. If we include the other 4 loci (i.e. AO-1, EST, GPDH-1, and LAP) and assume that they were all monomorphic (although AO-1, EST and GPDH-1 showed variations, they could not be clearly scored), we still have $P = 0.438$ and $H_a = 0.141$. This H_a value is still higher than reported for any hymenopteran species.

There are indications that some proteins are more likely to be polymorphic than others¹⁸⁻²⁰. It is possible that many of the enzymes we surveyed in our *M. nigripes* samples are generally more polymorphic than those surveyed in other hymenopteran species, and thus produced a higher level of heterozygosity in our analyses. However, a comparison of the enzyme systems used in our study and those of Menken¹⁷ and Shaumar et al.¹⁶ revealed that HK-1, HK-2, and SOD are polymorphic in *M. nigripes* but are monomorphic in the other 7 species of ichneumonid wasps. MDH is polymorphic in both *M. nigripes* and *Triclistus yponomeutae*¹⁷.

Ignoring *M. vittator* for which only 2 males and 1 female were used¹⁷, our isozyme survey in *M. nigripes* is the first report of genetic variability in a hyperparasitic hymenopteran. Environmental stability conferred by the microhabitat usually results in an increase in the effective population size of a species, and this in turn will contribute to the high levels of intraspecific genetic

variability^{15,21}. Our finding in this parasitic wasp seems to agree with this contention.

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The role of assortative mating among age-classes in *Drosophila melanogaster*: preliminary results

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Summary. A series of experiments on age-related mating success and productivity provides evidence for assortative mating among three out of four age-classes in *D. melanogaster*. The preferred mating does not always result in the highest productivity. Three age classes of males contribute to reproduction while only females of the youngest age-class are involved. The progeny size is more affected by the age of the females than that of males. It is assumed that these findings must have important implications for generation overlap in natural populations.

Key words. Age-structured population; age-related mating success; assortative mating; generation overlap; *Drosophila melanogaster*.

In a population where individuals belong to different cohorts, the question can be raised as to whether there is effective reproductive isolation or completely random mating among cohorts. Populations of many species are age-structured and the results of natural selection in such populations are of particular importance for the evolution of life-history phenomena such as senescence and age-related patterns of reproduction¹. In particular, the age-structure determines the frequencies of individuals subject to the age-specific fecundity and viability schedules².

The effect of aging on the mating capacity of male *Drosophila* has been investigated⁴⁻⁶. The mating capacity of males declines with advancing age. The decline is not uniform, however, and the age at which flies become infertile or are no longer able to mate shows considerable variations⁶.

The present work attempts to answer two questions: 1) Is there assortative mating among age-classes of *Drosophila melanogaster* and 2) if so, what are the consequences in productivity of 'like' and 'unlike' aged flies. The objective is to analyze the possible relation of these to generation overlap.

The *Drosophila melanogaster* strains used in the experiments were established from wild-caught (1983) females originating from the Taï rainforest in southwestern Ivory Coast (Gif 255.1). Eight outbred strains were similarly synthesized. Each of them originated from 136 virgin individuals, i.e., 4 females and 4 males produced by each of 17 isofemale lines. Individuals were reared on a standard axenic medium⁷ at 25°C. Under these conditions, we could observe up to five consecutive overlapping generations. Only the last four were used, however, owing to the small number of surviving individuals belonging to the oldest age-class when those from the youngest emerged.

Four identifiable age-classes were defined according to the generation span (about 13 days) and sexual maturation. These classes correspond to individuals aged 4, 17, 30 and 43 days.

A series of three independent experiments was carried out: 1) *multiple male experiment*: virgin males of the four age-classes were introduced into a 80 × 40 × 20 mm vial with virgin females of the youngest age-class (4 days old); 2) *multiple female experi-*

Table 1. Frequencies of mating pairs observed for a series of 8 replicated mating chambers in the multiple male experiment (1) and the multiple female experiment (2). Each mating chamber contained 16 ♂ and 16 ♀ in series of equal ratio and 28 ♂ and 28 ♀ in series of increasing and decreasing age-class ratios. Only the first four matings observed are recorded in each chamber whatever the density may be. Hence, the results for the 8 mating chambers apply to 32 matings only and not to the 128 possible in series of equal ratio or 224 possible in series of unequal increasing and decreasing ratios. The frequency of matings of each type observed in each experiment is summarized in the last column with respect to 96 possibilities instead of the 576 mating pairs that could be achieved

Age in days ♂ ♀	Equal ratio		Unequal increasing ratio		Unequal decreasing ratio		Total proportion of matings observed
	Number of ♂ ♀ per chamber	Proportion of matings observed	Number of ♂ ♀ per chamber	Proportion of matings observed	Number of ♂ ♀ per chamber	Proportion of matings observed	
Experiment 1: Multiple males							
43 4	4:4	4/32	4:7	1/32	10:7	2/32	7/96
30 4	4:4	10/32	6:7	10/32	8:7	2/32	22/96
17 4	4:4	13/32	8:7	12/32	6:7	14/32	39/96
4 4	4:4	5/32	10:7	9/32	4:7	14/32	28/96
Experiment 2: Multiple females							
4 43	4:4	1/32	7:4	0/32	7:10	0/32	1/96
4 30	4:4	5/32	7:6	0/32	7:8	4/32	9/96
4 17	4:4	1/32	7:8	3/32	7:6	7/32	11/96
4 4	4:4	25/32	7:10	29/32	7:4	21/32	75/96

Table 2. Number of mating pairs observed with males and females both belonging to four age-classes (experiment 3: combined). The individuals of each age-class are introduced to each mating chamber in equal ratio (4:4:4:4) in both sexes. Each chamber then contained 16 ♂ and 16 ♀. A series of 8 replicates is made and the results are given with respect to 32 matings that are recorded, as for experiments 1 and 2

Age of males (days)	Age of females (days)				Total
	43	30	17	4	
43	1	0	0	3	4
30	1	1	2	3	7
17	0	3	2	3	8
4	0	0	3	10	13
Total	2	4	7	19	32

ment: the reciprocal experiment; 3) *combined experiment*: four age-classes of both sexes.

For all three experiments, equal numbers of males and females were used and eight replications of the same experiment were done. To identify individuals of different age-classes, a microscopic wing clip was made. Flies were marked according to the class on either the left, right or both wings or were not marked. To avoid any artifact caused by the marking procedure age-classes were clipped in turn for the four marking types. In experiments 1 and 2, three series of ratios were used for individuals of different age-classes: equal ratio (4:4:4:4); unequal increasing ratio (flies 4, 17, 30 and 43 days old in ratio 4:6:8:10) and unequal decreasing ratio (flies 4, 17, 30 and 43 days old in ratio 10:8:6:4). In order to avoid bias due to the ratios used, only the first four matings to occur in each vial were noted. Each of the mating pairs was aspirated out of the vials, and maintained for 10 days in a tube containing standard axenic medium that was changed at 3–4-day intervals. The number of resulting offspring was recorded.

The results of the three experiments are given in table 1. In experiment 1, 17-day-old males consistently mated more fre-

quently than expected at each ratio. In the unequal decreasing and equal ratios, 30-day-old males were more successful than in the unequal ascending ratio. In contrast, mating success of 4-day-old males was enhanced for the unequal increasing ratio compared to equal and unequal decreasing ratios although their relative frequency was the lowest. In experiment 2, 4-day-old females were consistently the most successful age-class. Although based on relatively few observations the results of experiment 3 (table 2) are of interest. 4-day-old males and females are the most successful; although 4-day-old females mated with males of all ages, no 4-day-old males mated with 30- or 43-day-old females.

To test whether the mating pairs were formed at random in these experiments, the confidence intervals for each ratio were calculated according to a neobayesian approach⁸. For most mating ratios, the theoretical value, under a random mating hypothesis, is not included in the confidence interval calculated, hence supporting the conclusion of non-random association of the mating pairs.

Table 3 shows the number of offspring produced by the mating pairs observed in experiment 1 and 2. In both experiments, the progeny size decreases as the age of the mate increases. In spite of the large standard deviations, the differences between the means are significant when males or females are of non-consecutive age-classes. The differences between progeny size are less pronounced when females of the same age are compared for different age-classes of males. Nevertheless, the age of very old males can affect the progeny size. Fourteen virgin 69-day-old males and two 95-day-old males were crossed with young virgin 4-day-old females. These matings produced a somewhat reduced progeny size i.e. $\bar{X} = 119$ offsprings ($SE = 7.14$) for the first males and $\bar{X} = 160$ and $\bar{X} = 11$ offsprings for the last two males respectively. The age of the females appears to be more important, however, than the age of males in determining the progeny size. Indeed, an analysis of the effect of the age of virgin females at copulation on egg production shows that if copulation is delayed beyond 15 days then oviposition rate is reduced⁹.

In conclusion, these results provide clear evidence that 1) there is assortative mating among age groups, some age-classes being more successful than others and 2) the preferred mating does not always result in the highest productivity. Mating does not occur at random and the contributions of males and females are not similar. The youngest age-class of females and three age-classes of males are involved. Thus, for a *Drosophila* species showing an overlap of four generations under the experimental conditions used, only three of them appear contributive.

These conclusions may be limited by the necessity to distinguish between generations and age-classes and to be precise about what is meant by generations in the wild in more or less contin-

Table 3. Means and standard errors of the progeny size of the different mating pairs observed with multiple males (experiment 1) and multiple females (experiment 2)

Experiment 1					Experiment 2				
♂	♀	n	Age of		♂	♀	n	Age of	
			Mean	SE				Mean	SE
43	4	17	210.06	29.01	4	43	23	62.48	8.05
30	4	15	259.27	22.88	4	30	21	112.05	21.69
17	4	12	340.84	34.50	4	17	20	184.90	17.67
4	4	24	356.63	15.01	4	4	24	356.63	15.01

uously breeding species. Two mechanisms of generation overlap may exist; the first one involves individuals of a given age-class but of different generations that eclose synchronously (generation overlap within an age-class, which is necessarily between cohorts); and the second one involves newly eclosed females of the youngest age-class and males of the older age-classes from other generations (generation overlap between age-classes regardless of the cohort). The relative importance of these two mechanisms depends on both the proportions of the different generations and the duration of adult eclosions within each of them.

Numerous authors have described male mating success as an age-dependent phenomenon. It increases to a plateau value in the first days of life (6–8 days) and decreases a few days after the plateau has been reached^{5, 10, 11}.

If the results obtained on age-related mating success and productivity are important for understanding mate 'choice', the relevance of the findings for natural populations is that older males have a mating advantage during the short period when newly eclosed females are sexually mature and no newly eclosed males are 'available'. This situation is likely to occur in populations living in temperate places especially when they 'start up' each spring.

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Responses of the cavefish *Astyanax mexicanus* (*Anoptichthys antrobius*) to the odor of known or unknown conspecifics

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Summary. Juvenile individuals of the cave characid *Astyanax mexicanus* recognize to a significant extent, in a choice-apparatus, the odor of known conspecifics as compared to that of unknown ones during the first 2 min of a 10-min experiment. After this initial oriented response, exploration becomes random.

Key words. Cavefish; blindfish; specific odor; chemical sense; chemoreception; locomotory response; choice reaction.

Several studies¹ have stressed the biological significance of chemoreception in the behavior of cavefishes, and its dominant role in the detection at a distance of the odor of food and of conspecifics. Concerning the detection of the latter, Berti et al.^{2, 3} were able to show that samples of water drawn from home-tanks occupied during a given length of time (2–10 months) by adult individuals of the cavefish *Caecobarbus geertsi* (9 individuals) or *Phreatichthys andruzzii* (13 and 14 individuals) were characterized by the presence of chemical traces, able to attract systematically a test-fish of the same species in an appropriate choice-apparatus. Results show, moreover, that the test-fish tends to orient itself preferentially in the direction of these traces, be they those of known or unknown conspecifics. In a more recent study, de Fraipont⁴ showed that juvenile individuals (total length: 40 mm) of the blind characid *Astyanax mexicanus* (formerly *Anoptichthys antrobius*) displayed the same preferential locomotory response to the odor of conspecifics and that the

fish, whatever its home-group was, was always more attracted by the chemical traces of the larger group. Since home-groups with different numbers of individuals were always kept in the same volume of water, the intensity of the response is a function of the concentration of chemical traces left behind by the home-individuals in the water. In the above-quoted experiments of Berti et al., the test-fish had to make a choice between a sample of water drawn from a home-aquarium containing conspecifics, and distilled water. In the present experiments, a further problem was tackled. Given the fact that a test-fish reacts positively to the odor of conspecifics as opposed to pure water, an investigation was made of whether it would discriminate between the odor of a home-group to which it belonged itself and the odor of unknown conspecifics.

A total of 120 juvenile *Astyanax mexicanus* (*A. antrobius*) total length: 47 mm) of the same hatching were used. Eight days after hatching, they were distributed in 4 × 2 groups of 4 (4A–4B), 8 (8A–8B), 16 (16A–16B) and 32 (32A–32B) individuals. All groups were kept in the same volume of water (48 l) during 8 months. Two groups (A and B) were necessary for each density in order to have an equal number of known and unknown conspecifics available in each case. The experimental trough was

Table 1. Example of experimental design for a test-fish belonging to the 32A home-group. This design was applied in all groups

Home group of test-fishes (6 sessions per group)	Origin of water sample at right end of trough (3 sessions each)	Origin of water sample at left end of trough
32 A	32 A or B	32 A or B
32 B	32 A or B	32 A or B
16 A	16 A or B	16 A or B
16 B	16 A or B	16 A or B
8 A	8 A or B	8 A or B
8 B	8 A or B	8 A or B
4 A	4 A or B	4 A or B
4 B	4 A or B	4 A or B

Table 2. Statistical analysis (χ^2) of results obtained during the two first min of registering of test-fish positions

Groups tested	Class frequencies			χ^2	p
	Positive	Neutral	Negative		
32 known → 32 unk.	9	1	2	4.45	0.05 < p < 0.02
16 known → 16 unk.	10	0	2	5.3	0.05 < p < 0.02
8 known → 8 unk.	10	0	2	5.3	0.05 < p < 0.02
4 known → 4 unk.	11	0	1	8.33	0.001 < p < 0.01