Zeitschrift: Mitteilungen der Schweizerischen Entomologischen Gesellschaft = Bulletin de la Société Entomologique Suisse = Journal of the Swiss Entomological Society

Herausgeber: Schweizerische Entomologische Gesellschaft

Band: 69 (1996)

Heft: 2

Artikel: Mating variables in three native Chymomyza species of Switzerland (Diptera : Drosophilidae)

Autor: Burla, H. / Stahel, W.

DOI: https://doi.org/10.5169/seals-402627

MITTEILUNGEN DER SCHWEIZERISCHEN ENTOMOLOGISCHEN GESELLSCHAFT
BULLETIN DE LA SOCIÉTÉ ENTOMOLOGIQUE SUISSE
69, 173 – 187, 1996

Mating variables in three native *Chymomyza* species of Switzerland (Diptera: Drosophilidae)

H. BURLA[1] & W. STAHEL[2]

[1]Zoological Museum, University of Zurich-Irchel, Winterthurerstrasse 190, CH-8057 Zurich, Switzerland.
[2]Statistical Seminar, ETHZ, CH-8092 Zurich, Switzerland.

In three species of *Chymomyza*, paired flies were kept in observation cells and videorecorded for about 3 hours. Three mating characteristics were measured: proportion of copulating pairs, time to first copulation (TC1) and duration of first copulation (DC1). The measurements involved 192 pairs, namely 123 of *Chymomyza costata*, 53 of *C. fuscimana* and 16 of *C. distincta*. There was a strong variation in all mating characteristics. In *C. costata,* the variation between a stock from the midland and another one from the alps suggests incipient isolation.

Keywords: mating, time to copulation, duration of copulation, remating, incipient isolation.

INTRODUCTION

Of 5 *Chymomyza* species known to occur in Switzerland, the semi-domestic *C. amoena* is a recent immigrant from the U.S.A. (BURLA & BÄCHLI, 1992; MACA & BÄCHLI, 1994; BAND, 1995a) whereas the others, *C. costata, C. caudatula, C. distincta*, and *C. fuscimana*, are native. The native species are most easily seen in forests on the exposed wood of freshly damaged trees (BURLA, 1995a, 1995b). *C. amoena* may be found in forests but also in semi-domestic open habitats (BURLA & BÄCHLI, 1992; BAND, 1995b).

Mating characteristics are of interest in evolutionary biology. They vary among and within species as much as morphological characters do. They may measure the degree of evolutionary isolation between closely related taxa such as sibling species, subspecies or races. In areas where two or more such units are sympatric, their further existence depends on reproductive isolation. The easiest way of checking for isolation is to see whether they still mate, and in how large a proportion. A demanding means is to analyze and compare elements of courtship behavior. It is simpler, and to some degree sufficient, to measure only three variables: the proportion of copulating pairs, the time to copulation (TC) and the duration of copulation (DC), as done in the present report. Low proportion of copulating pairs and long TC indicate poor mating performance and may point to incipient sexual isolation. In *D. melanogaster,* time to copulation (mating speed) differences were observed between two isofemale stocks from places only 100 miles apart (HOSGOOD & PARSONS, 1965). In *D. subobscura*, TC and DC varied between stocks from four places in Chile (OCHANDO *et al.*, 1991).

Beginning in the eighties, TC and DC were measured in *Chymomyza* species from the U.S.A. (BAND, 1995b). Comparing six stocks of *C. amoena*, mean TC ranged from 8.2 min. to 14.1 min. and mean DC ranged from 14.7 min. to 22 min. (BAND, 1995a). Mean DC differed significantly between two stocks derived from

different places in Michigan, U.S.A. as well as between two stocks from the Maggia Valley, Switzerland, one stock being derived from flies netted over bait, the other stock from flies which had emerged from acorns and chestnuts. In the present report, the measurements of TC and DC are extended to a fairly large sample of *C. costata* and smaller samples of *C. fuscimana* and *C. distincta*, from stocks which had been grown from flies collected in two places of Switzerland, one in the midland, the other in the alps.

These mating characteristics are known to be subject to experimental influence by a variety of conditions. In *D. melanogaster*, STURTEVANT (1915) checked for variations of TC that could be traced to an influence of visual and olfactory stimuli, to movements of the wing and to mutant phenotypes. Heritability of TC was demonstrated by MANNING (1961). Differences in TC were controlled by the strain of the male rather than of the female (HOSGOOD & PARSONS, 1965). In *D. pseudoobscura*, DC and TC varied between inversion karyotypes (KAUL & PARSONS, 1965; PARSONS & KAUL, 1966). TC appears to be a major component of fitness (PARSONS, 1974). In *D. subobscura*, TC and DC were seen to vary between karyotypes (GLATTHAAR & SCHENKER, 1972; OCHANDO *et al.*, 1991).

The fact that mating characteristics vary within and among stocks is relevant in connection with a current dispute on the evolution of species (BAND, 1995b). In a Recognition Concept of speciation, PATERSON (1993) presumed that, under the pressure of stabilizing selection, the system of courtship behavior, involving signals and responses, becomes invariant among members of a local population in their natural habitat, which characterize the species as a whole. Yet he accepts that the behavioral system may undergo changes if a population of the species moves to a new habitat. In other words, there may be variation between specimens from separate habitats but not within the natural habitat. Many observations, however, provide evidence for both components of variation.

Although the variables TC and DC are not elements of courtship behavior in the narrow sense, they measure the outcome of the courting process.

MATERIAL AND METHODS

Flies

In both *C. costata* and *C. fuscimana*, a midland stock from Gockhausen and an alpine stock from Tinizong were used. Collecting sites are described by BURLA (1995a, 1995b). In *C. distincta*, a stock from Finland (obtained by courtesy of Dr. S. LAKOVAARA) and another from Gockhausen were used. The stocks were maintained in population cages. The culture medium was prepared according to a recipe by Dr. S. LAKOVAARA (pers. comm.), using locally available ingredients. Other flies used for the present study were freshly captured or reared from preadult stages found in nature.

The founders of the *C. costata* Gockhausen stock were 3 females and 6 males, captured between July 13 and 24, 1994, at two forest places 750 m apart. After 5 months there were about 100 flies in the stock. The founders of the *C. costata* Tinizong stock were 6 females and 13 males, captured on June 23 and 24, 1994, on two fir stumps 2 m apart, in a vast alpine forest. Population density increased very fast, reaching about 200 flies within two months. A small proportion of emerged flies were of dwarf size, suggesting shortage of food at the larval stage. Their presence allowed for efficient testing of body size as a factor in mating. In *C. fusci-*

mana, body size varied so little that this variable was excluded from statistical testing.

Freshly emerged flies were matured before being paired by keeping them separate by sex, often singly, in culture vials. The range and mean of number of days of maturation are mentioned in Tab. 9, separate by species and sex.

The pairs were videorecorded in groups of four. In most pairs of *C. costata*, partners from stocks Gockhausen (Go) and Tinizong (Ti) were combined according to the following scheme:

	male Go	male Ti
female Go	a	b
female Ti	c	d

In cells a and d, the partners are homologous with respect to the origin of the stock founders from a locality while in cells b and c, they are heterologous. In *C. fuscimana*, due to a shortage of flies emerged from the two stocks the above scheme could be realized in only 7 out of 13 runs. In the other runs, emergees from a Finland stock were used, or adult flies collected in nature, or reared from larvae collected under bark (Tab. 9).

Part of the pairs of *C. fuscimana* were conditioned to mating by keeping each female and male in a flat part of a subdivided cell (Tab. 9). The subdivisions were separated by a veil which allowed visual, olfactory and acoustic signals to pass. A small amount of mashed apple was accessible to the flies for feeding.

Equipment

For videorecording the following material was used: a Sony SVO-9500MDP recorder; a remote control unit SVRM-100; a Panasonic WV-CM1000 colour monitor; a fiberoptic double lamp 12V/100W; VHS cassettes, 8 each of 180 minutes (lasting 3 to 4 minutes longer) and 40 of 195 minutes (lasting 2 to 3 minutes longer). The control unit allowed playback of the recordings at variable speeds, down to a slow sequence of single pictures, and standstill. Thus, timing was accurate.

Observation cells

A series of cells were carved out of white PVC by a computer controlled industrial machine. Weeks before their first use they were sprayed with light grey Dupli-Color primer HG01-132636. The cells measure 31 x 27 mm at the top and are 8 mm deep. The side walls are a little inclined. The cells are covered by a transparent colorless acryl glass. Four such cells were simultaneously used in each recording. The flies were transferred from a culture vial to the observation cell by means of an aspirator, and paired on this occasion. They were introduced by a hole in the cell bottom, 8 mm in diameter. After every use with a pair, cells and glasses were thoroughly washed with soap and repeatedly rinsed with water, then dried in hot air and stored for a day. The temporal and optical resolution was good enough to assess the behavior of the flies, as relevant to the task, without error.

The study was carried out during winter 1994/95. Culture vials containing pupae and emerging flies were kept in a study room with constant temperature of 19 °C , constant relative humidity of 60 % and the natural cycle of daylight. Videorecordings were carried out in the same room. Flies were paired from morning to afternoon. Late recordings extended into night.

Evaluation of the experimental setup

The data obtained suggest that the experimental setup was sufficient for a majority of pairs to copulate. Yet the flies no doubt were disturbed by the transfer and often kept circling along the edge of the cell, trying to escape. After recognizing the presence of another fly they changed erratically between escaping behavior and courtship. *C. distincta* gave the impression of being more upset by the confinement than the other species, spending more time on trying to escape. Thus, conditions for mating were far from normal.

It turned out that three hours of recording were not enough to account for all copulations. In spite of this, videorecording time was not increased, mainly because there was no means to offer food, drink and fresh air to the flies in the cell. It was feared that with prolonged recording under this condition, fly behavior might deteriorate.

Mating variables

The following measurements were taken:

– TCi, time in minutes before copulation i. i varied from 1 to 4. The time was measured from the begin of recording in the case of TC1, and from the end of DC(i-1) in the case of rematings.

– DCi, duration in minutes of copulation i.

– SDC, sum of all DCi values noted in a cell during about 3 hours.

The names TC and DC were chosen in preference to TM and DM as used by BAND (1995a) where M means mating. PARSONS (1974) and OCHANDO *et al.* (1991) used s (mating speed) for TC. LONG *et al.* (1980) split TC into courtship latency (in seconds) and courtship duration (in minutes).

Statistics

Whereas all males courted the female, there were females which refused copulation until the video cassette was exhausted. The question is how to deal with these cases. One option is to consider copulating pairs only, as, apparently, PARSONS (1974), OCHANDO *et al.* (1991) and BAND (1995a) did. In the present case, this procedure would exclude pairs which might have copulated after the 3 hours of videorecording, and would ignore the possibility that pairs would never copulate. The problem is adequately treated by Cox's regression model which is fitted by the program BMDP2L. The program enters the independent variables one by one into the multiple regression equation in decreasing order of their effect, and removes those again which prove insignificant in the equation. The program was run separately for TC1, DC1 and SDC in turn. Because the three variables are correlated (Tab. 3), the tests are redundant to some degree.

The scheme of combining partners from the same or different localities, as shown above, was accounted for by three dummy variables. To check for an effect of male origin, a dummy variable "LMAL" was given value 1 in cells b and d, otherwise zero. Similarly, "LFEM" was given value 1 in cells c and d, otherwise zero. Finally, "LINT" was given value 1 in cell d, otherwise zero.

The decision whether to include, or exclude, non-copulating pairs had to be made in other parts of the data analysis too. When computing elementary statistics (Tab. 1), non-copulating pairs were disregarded. The effect was to lower TC1 values while increasing both DC1 and SDC values, making the results better compa-

rable to some previous estimates from literature. Non-parametric correlation coefficients (Tab. 3) were based on complete data. For this purpose, in non-copulating pairs TC1 was set equal to 190 min. Both options were used when computing coefficients of variation (Tab. 4).

In *C. fuscimana*, DC1 was excluded from part of the statistical analysis as the measurements were equal to SDC in most pairs.

RESULTS AND DISCUSSION

Proportion of copulating pairs

In both *C. costata* and *C. fuscimana,* most of the pairs copulated, while in *C. distincta,* only about half of the pairs did (Tab. 1). In a similar study involving *D. subobscura* (GLATTHAAR & SCHENKER, 1972), the proportion of mating pairs varied between karyotypes and depended on the light intensity. On the average, it was 52%. It was as low as 6.5% in Chilean stocks of the same species, yet it was higher if the paired flies were from the same stock than from different stocks (OCHANDO *et al.*, 1991).

Time to first copulation (TC1)

The first copulation (Fig. 1, Tab. 1) was instantaneous (TC1 = 0 min.) in one pair of *C. costata* and almost instantaneous (TC1 = 1 min.) in 13 pairs of *C. costata* and one pair of *C. fuscimana* (TC1 = 1). Mean time to first copulation was lowest in *C. fuscimana*, highest in *C. distincta* and intermediate in *C. costata.* If medians are compared instead of means, *C. distincta* was again slowest to mate while the other two species exchanged rank orders.

The observed means are larger than those reported for stocks of *C. amoena* and *C. aldrichii* (BAND, 1995a, 1995b). They are larger than in *D. melanogaster* (HOSGOOD & PARSONS, 1965; LONG *et al.*, 1980) and *D. pseudoobscura* (KAUL & PARSONS, 1965; PARSONS, 1974). Finally, they are somewhat larger than in two wild *Drosophila* species from Brazil, *D. schineri* and *D. eleonorae* with mean TC equal to 25.7 and 8.8 minutes, respectively (MAPELLI & VILELA, 1994; SHOJI & VILELA, 1994). Yet, such comparisons are hampered by the fact that values are influenced by the technical set-up which usually varies between authors.

Whereas DC as measured in the laboratory may reliably represent natural behavior, the situation is different in the case of TC. In the wild it seems that females come to a copulation site only if they are prepared to copulate (BAND, 1995b). How long a female waits before she approaches a male, or is recognized by him, is unknown. Isolated immobile females were sometimes observed not far from the copulation site or at its rim. Hence TC, as measured in the present study, is an artifact, created by uniting pairs of flies in a small observation chamber. In a forest, the corresponding event is not measurable. However, even in captivity TC must depend on the natural mating system of the species. Hopefully, it is proportional to the mating propensity of the flies.

Very low TC1 values (of zero or a few minutes) suggest that conspecificity was recognized almost immediately at an encounter. It could mean that little or no element of an elaborate courtship behavior was operative. If, however, courtship is prolonged and all its elements are displayed time and again, its function is rather to increase readiness for copulation in the female, and perhaps to trigger physiologi-

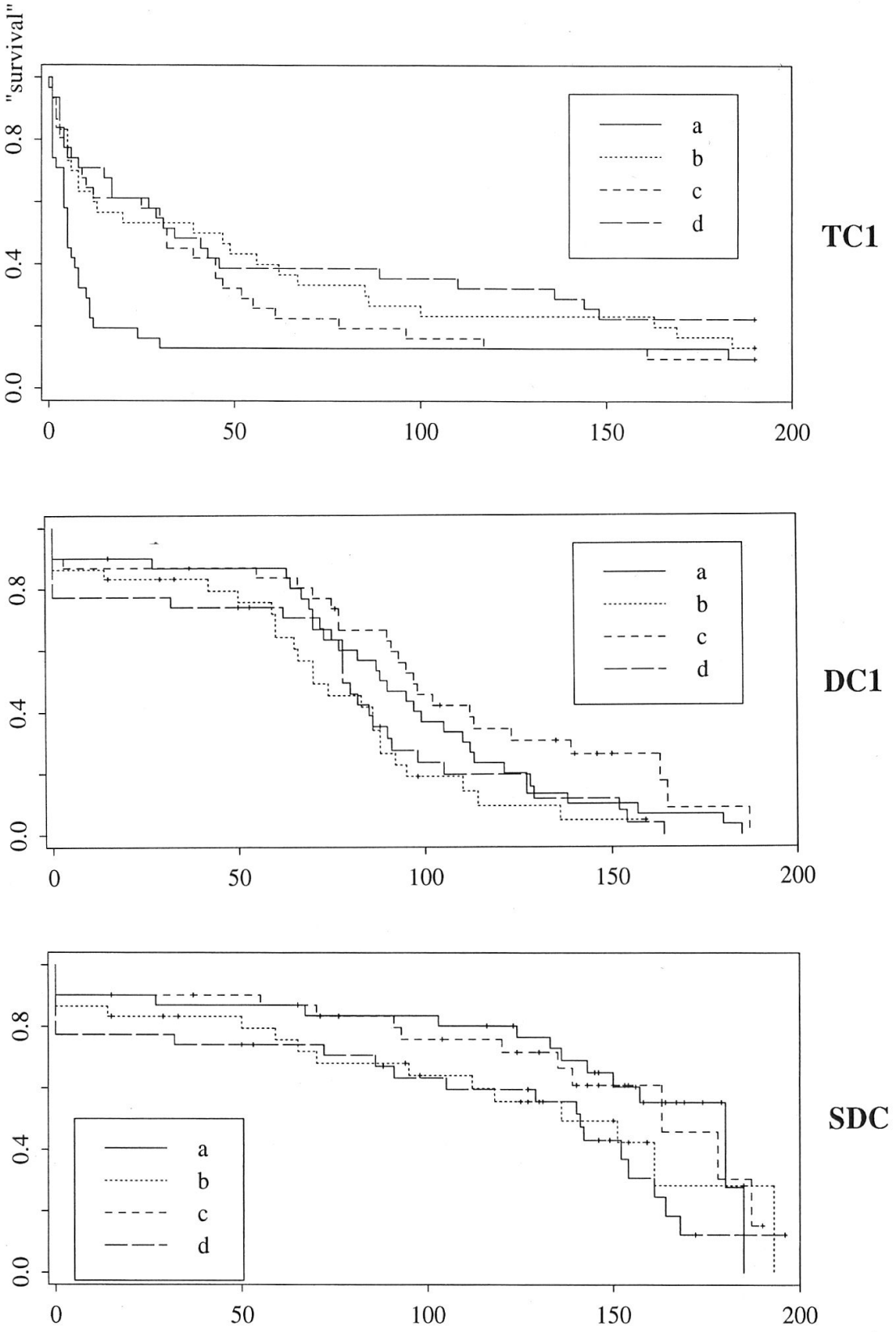

Fig. 1. Survival curves characterizing the distributions of TC1, DC1 and SDC in *C. costata*, separate by the four cells a to d. For each value t (on the abscissa) the curves show the proportion within the cell groups with the respective variable > t. For TC1 and t=50, for example, the proportions are 0.12, 0.42, 0.31, and 0.39 for cells a to d, respectively.

Tab. 1. Elementary statistical values, separate by subsequent copulations. TC, time to copulation, DC, duration of copulation, both measured in minutes.

		Chymomyza costata		*Chymomyza fuscimana*		*Chymomyza distincta*	
	nr of pairs	123		53		16	
	nr of copulating pairs	106		45		9	
	percent pairs copulating	86		85		56	
		TC	DC	TC	DC	TC	DC
1st copula	N	106	106	45	45	9	9
	Min	0	3	1	1	6	17
	Max	184	187	172	109	76	48
	mean	33.19	91.7	27.16	23.51	36.8	28.4
	SE	4.44	3.71	6.17	2.34	9.83	2.94
	median	10.5	88	12	21	24	27
2nd copula	N	62	62	3	3	1	1
	Min	1	1	15	2		
	Max	108	83	25	45	90	2
	mean	23.5	47.29				
	SE	2.7	2.74				
	median	17.5	52				
3rd copula	N	18	18	2	2		
	Min	1	1	14	11		
	Max	38	55	61	26		
	mean	14.11	28.72				
	SE	2.95	4.58				
	median	10.5	31.5				
4th copula	N	3	3	2	2		
	Min	6	5	20	1		
	Max	40	22	25	9		
	mean	23	12.67				
	SE	9.81	4.98				
	median	23	11				
total	N	106		45		9	
	Min	14		6		17	
	Max	196		122		48	
	mean	124.57		26.29		28.67	
	SE	4.36		3.23		2.98	
	median	136		21		27	

cal processes which are needed to prepare the transfer and storage of sperm. This may delay a female's acceptance of a mating partner.

Duration of first copulation (DC1)

In Tab. 1, the measurements of DC1 are given separately for subsequent copulations. In *C. costata*, mean DC was highest in the first copulation. It diminished by a factor of about 2 for every next copulation. The longest first copulation lasted a little longer than three hours and, in *C. fuscimana*, a little less than 2 hours. Compared to other *Chymomyza* species, the present mean DC value of *C. costata* (91.7 min.) is exceedingly high. In the American *C. procnemoides*, it was 32.8 min. in 39 pairs while in *C. aldrichii,* it was 12.4 min. in 11 pairs (BAND, 1995b). In *C. amoena*, the highest mean DC was 20.5 min. in 20 pairs from a Michigan population and 22.0 min. in 21 pairs from a Swiss population (BAND, 1995a). In *D. subobscura*, mean DC values varied between 13 and 21 min. among 8 groups involving 859 pairs in total (GLATTHAAR & SCHENKER, 1972). In *D. melanogaster*, mean DC values

Tab. 2. Frequency distribution of DC values grouped into intervals of 10 minutes. Separate for the first copulation (DC1), additional copulations (DC2 to DC4) and SDC (sum of DCi). In some columns the frequency distribution appears to be roughly normal, save for the frequencies of class DC = 0. Other distributions are skewed to the origin.

	Chymomyza costata					*Chymomyza fuscimana*					*Chymomyza distincta*		
class	DC1	DC2	DC3	DC4	SDC	DC1	DC2	DC3	DC4	SDC	DC1	DC2	SDC
0	17				17	8				8	7		7
1	1	7	5	1		20	1	1	1	1		1	
2	3	3	0	1	3	12			1	19	1		1
3	2	4	3	1	2	2	1	1		17	4		4
4	3	3	4		3	2				5	3		3
5	3	10	2		2	3	1				1		1
6	5	13	4		3								
7	13	18			4	2				1			
8	14	3			3								
9	15	1			2								
10	13				6	1							
11	6				4					1			
12	5				4								
13	6				10					1			
14	5				12								
15	2				14	1							
16	4				13								
17	3				10	1							
18	1				5	1							
19	2				4								
20					2								
sum	123	62	18	3	123	53	3	2	2	53	16	1	16

range around 20 min. (HOSGOOD & PARSONS, 1965). In the two Brazilian wild species mentioned before, mean DC was 13.3 and 3.2 minutes, respectively (MAPELLI & VILELA, 1994; SHOJI & VILELA, 1994).

In Tab. 2, the frequency distribution of DC values is grouped in intervals of 10 minutes, separate by species and subsequent copulations. Obviously, there is strong variation. Contrary to this, BRESSAC *et al.* (1991) reported DC to be relatively stable among matings for each species then involved, which were *D. affinis*, *D. latifasciaeformis* and *D. littoralis*. Similarly, OCHANDO *et al.* (1991) observed only small variation between mean DC values in *D. subobscura*. In *D. pseudoobscura*, mean DC varied within narrow limits but strongly enough to suggest a heterokaryotype advantage, restricted to males (KAUL & PARSONS, 1965; PARSONS & KAUL, 1966). It is obvious that the observed variation not only dependeds on the genus and species of the flies, but also on the origin and age of the stocks, and to what degree the flies were inbred.

According to MACBEAN & PARSONS (1967), PITNICK (1991), and BAND (1995a, 1995b), duration of copulation is primarily determined by the male. This statement is based on statistical analysis of data of pairs from the same or different geographical origin. However, in the present case the impression prevailed that most of the time the female interrupted the copulation. The following sequence was observed repeatedly. At the start of a copulation, the female usually keeps moving, dragging the male behind her. Shortly thereafter she slows down and eventually becomes immobile, resting at an edge of the cell where the pair stays immobile during most of the copulation. Eventually the female begins to move again. Shortly later the partners separate. At slow motion at this point it looks as if the female

Tab. 3. Spearman correlation coefficients between the dependent variables TC1 (time to first copulation), DC1 (duration of first copulation), SDC (sum of all DCi) and NCOPS (number of copulations during the 3 hours of videorecording). Based on 123 pairs of C. costata. The critical value of a Pearson correlation coefficient is 0.177 at p=5 % and 0.232 at p=1 %, for d.f. = 121. Hence, all values in the table differ significantly from zero. In *C. fuscimana*, the Spearman correlation coefficient between TC1 and SDC is equal to -0.233, as computed from 53 cases. The critical value is 0.271 for d.f. = 51.

	TC1	DC1	NCOP
DC1	–0.559		
NCOP	–0.645	0.288	
SDC	–0.732	0.780	0.629

would disconnect her mate. After being thrown off, the male seems to be startled for a while, whereupon he resumes courting. Ardent courtship by the male after a separation from the female is a rule with almost no exception in all three species. It too suggests that it was the female, not the male, which interrupted the copulation.

Comparing TC1 and DC1

Spearman correlation coefficients between the measured variables TC1, DC1, SDC and the number of copulations per pair (NCOP) are highly significant in *C. costata* (Tab. 3). As expected, TC1 is negatively correlated with the other three variables. It was negatively correlated with DC also in *D. pseudoobscura* (PARSONS & KAUL, 1966). In *C. fuscimana*, the correlation between TC1 and SDC is not significant.

A general question is whether DC is less variable than TC (BAND, 1995a). Comparing the coefficients of variation of the two measurements (Tab. 4), the answer is yes in the present case. In *D. subobscura*, OCHANDO *et al.* (1991) obtained considerable variation only with TC, not DC. In *D. melanogaster* and *D. pseudoobscura*, mean DC values were rather constant but varied with temperature (PARSONS & KAUL, 1966). This may be caused by using flies from inbred stocks. In the present case, flies were not inbred and thus more representative for behavior in nature. Yet, it may be that TC1 varies strongly because of its artificial nature while DC1 is less affected by the confinement.

Tab. 4. Coefficient of variation, CV = SD/mean. Noncopulating pairs were excluded in the upper three rows but included in the lower two. In the upper four rows, TC1 varies more than DC1.

Chymomyza	non-copulating pairs	TC1	DC1	SDC
costata	excluded	1.379	0.416	0.365
fuscimana	excluded	1.524	0.667	0.824
distincta	excluded	0.802	0.311	0.312
costata	included	1.257	0.602	0.562
fuscimana	included	1.534		0.901

Tab. 5. Number of pairs which were casually seen in copulation after the end of videorecording when they were kept in empty vials until their mesonotum was measured. The proportion of remating pairs differs between species; it is highest in *C. costata*. The last copulation (not mentioned in the table) was noticed 19 hours after the begin of videorecording.

		hour after begin of videorecording							
Chymomyza	pairs	4	5	6	7	8	9	10	11
costata	present	123	123	100	48	31	27	7	
	in copula	64	58	39	19	7	6	2	1
fuscimana	present	49	44	20	12	8			
	in copula	2	2	0	0	1			
distincta	present	12							
	in copula	1							

Rematings

During the 3 hours of videorecording, 44 out of the 106 copulating pairs of *C. costata* copulated only once, 45 twice, 14 three times and 3 four times. The corresponding numbers were 42, 1, 0, 2 in *C. fuscimana* and 8, 1, 0, 0 in *C. distincta*. In all three species, rematings became successively shorter. In *C. costata*, mean time to copulation (TCi) changed only little between subsequent copulations. In *C. fuscimana*, TC1 and TC2 were equally short but TC3 was rather long.

Additional copulations were seen when after the end of the 3 hour recording period the flies were transferred to empty vials and left there until the mesonotum was measured. During the waiting time which ordinarily lasted between one and a few hours, flies continued to copulate. Some of the copulations were noted (Tab. 5), the others, which might have been the majority, ignored. In addition to the *C. costata* pairs mentioned in the table, 3 out of 4 pairs accidentally kept in empty vials were in copulation 18 hours after being joined for videorecording, and 2 out of 4 pairs after 19 hours. The data demonstrate that *C. costata* flies remated more often and longer than the other two species.

Remating has been discussed by BAND (1995a, 1995b). She observed it to be more frequent in *C. procnemoides* and *C. aldrichii* than in *C. amoena*. It seems to be common in *Chymomyza* with incidence varying between species. In *D. subobscura*, a number of males resumed courting after a copulation had ended (GLATTHAAR & SCHENKER, 1972). Their proportion varied between karyotypes, and it was higher if the first copulation was rather short than long.

Variation between stocks

Mating performance may be expected to differ between the stocks, Gockhausen and Tinizong, in one or both sexes. Homologous pairs (partners from the same stock) may be expected to perform better than heterologous pairs (partners from different stocks). Good performance is suggested by a high proportion of copulating pairs and short TC. It is less easy to intuitively assess the meaning of DC. According to PARSONS & KAUL (1966), short DC is selectively advantageous. However, from watching pairs in the arena, long DC is suggestive of good male performance.

Tab. 6. Mating variables separate by each of four observation cells a to d, arranged as shown on p. 175. In *C. costata*, 31, 30, 31, 31 pairs per cell were videorecorded, respectively, in *C. fuscimana*, 14 pairs per cell. The proportion of copulating pairs (percents rounded to the nearest integer) relates to these numbers. In the computation of mean TC1, DC1 and SDC, non-copulating pairs were omitted. In *C. costata*, the numbers of copulating pairs were, respectively, 28, 26, 28, 24, and in *C. fuscimana*, they were 13, 8, 13, 11. In *C. distincta*, the numbers of pairs per cell were too small for reliable comparisons.

Chymomyza	% copulating pairs		mean TC1		mean DC1		mean SDC	
costata	90	87	12.68	46.08	96.82	74.69	138.14	107.88
	90	77	35.89	40.00	102.89	91.04	125.96	125.17
fuscimana	93	64	42.31	18.13			40.85	22.25
	100	85	25.38	17.91			19.69	19.82

In *C. costata*, the proportion of copulating pairs (Tab. 6) was highest among pairs containing a Gockhausen stock male, and lowest among homologous pairs from Tinizong. Comparing this proportion with the proportion in the pooled data from all pairs in a contingency table, the heterogeneity is not significant. In *C. fuscimana*, the performance was poorest in cell b. Again, the hypothesis of heterogeneity cannot be rejected because of the low frequencies involved. In *D. subobscura* from Chilean stocks, the proportion of copulating pairs was higher in homologous pairs than heterologous ones (OCHANDO *et al.*, 1991).

In *C. costata*, mean TC1 was smallest (best performance) in cell a, but it was large in cell d, and while mean DC1 was large (good performance) in cell a, it was even larger in cell c (Tab. 6). However, mean SDC was again best in cell a, as expected. In *C. fuscimana*, mean TC1 was longest (poor performance) in cell a and shortest in cell d, just the opposite as in *C. costata*. Yet, mean DC1 was longest (best performance) in cell a. Hence, results in Tab. 6 are inconsistent.

Information on performance may be extended over the entire range of the respective variable (Fig. 1). Whereas in DC1 and SDC the survival curves of the *C. costata* pairs are similar in all four cells, in TC1 the curve representing cell a drops at the origin faster than the three other curves. According to a log-rank test, curve a significantly differs from curve b ($p=0.031$), curve c ($p=0.046$) and curve d ($p=0.005$).

Statistical effects of stock origin on performance are furthermore demonstrated in Tab. 7. Males from stock Gockhausen were, on the average, faster to copulate than males from stock Tinizong (negative sign with LMAL, $p<0.05$), and stayed longer in copulation than these (positive sign with LMAL, $p<0.05$). Similarly, females from stock Gockhausen were faster to copulate than females from stock Tinizong (negative sign with LFEM, $p<0.05$). The effect of origin of the stock was stronger in males than in females.

The term LINT is not mentioned in Tab. 7, meaning that an interaction between male and female stock origin was not statistically significant. The outcome was better if, after screening the raw data, four pairs with extreme TC values were disregarded in each of the four cells a to d. In the frequency distribution of TC1 (Fig. 1), the dropped cases are at the tail where the slope is minimal and thus of little importance. Now all three dummy variables are significant (Tab. 8). The regres-

Tab. 7. Results of a stepwise multiple regression contained in a life table analysis (BMDP2L), carried out on 123 pairs of *C. costata* and 53 pairs of *C. fuscimana*. Covariates which the procedure entered into the equation without removing them again, are listed in the third column (the variable names are explained in Tab. 9). The Chi square (d.f. = 1) in the 5th column relates to the improvement of the regression equation brought about by the respective covariate. The sign of the coefficient (4th column) indicates in which way the covariate affected the dependent variable.

Chymomyza	TIME=	covariate remaining in equation	sign of effect	improvement X2	p
costata	TC1	LMAL	–	5.24	0.022
		MSOC	–	4.61	0.032
		LFEM	–	4.18	0.041
	DC1	LMAL	+	6.78	0.009
		HOUR	+	3.20	0.073
	SDC	FSOC	+	4.87	0.027
		LMAL	+	3.41	0.065
fuscimana	TC1	MLOC	-	7.39	0.007
	SDC	MMAT	+	4.93	0.026
		MLOC	+	8.02	0.005

sion sign of LINT suggests that, on the average, homologous pairs performed better than heterologous ones.

In *C. fuscimana*, males from Gockhausen had shorter TC1 and longer SDC than males from Tinizong (MLOC in Tab. 7).

In conclusion, mating variables are influenced by the stock from which one or both flies of a pair were taken. In general, performance was better in homologous pairs than heterologous ones, suggesting incipient isolation.

Whether differences between stocks from Gockhausen and Tinizong point to geographical variation between the respective natural populations, or are due to unknown properties which developed in the stocks later, is open to question. The distance between the two localities is 116 km as the crow flies. This is little to overcome. Time and again a small proportion of flies will passively travel from the midland to the alps, or the other way round, when blown away by wind or carried on the ground while hidden in logs in preadult stages. Yet the two places differ by habitat and climate. For instance, the proportion of deciduous trees is larger at Gockhausen than Tinizong. At Tinizong the winters are more severe than at Gockhausen. To date, evidence for genotypic differences between the respective natural populations is lacking. If, on the other hand, stocks are considered instead of natural populations, founder effect cannot be excluded. Also, it is unlikely that the breeding conditions in the two population cages were identical, as most of the time the population density was higher in one stock than the other.

Effects of covariables other than the origin of stock (Tab. 9)

In *C. costata*, the more flies were kept in a vial for maturing jointly, the shorter TC1 became with males, while the longer SDC became with females (MSOC and

Tab. 8. Dummy variables as used in another run of Cox's model of regression for survival data, for assessing the effect of stock origin (see table 9, LOC) of paired flies. LMAL, origin of male, LFEM, origin of females, LINT, interaction between flies of different origin. coef, regression coefficient, se, standard error. In each of the four cells, the four cases with the largest TC1 values had been discarded, leaving 104 pairs which copulated. Likelihood ratio test = 25.4 on 3 d.f., p=0.000.

dummy variable	coef	se(coef)	p
LMAL	–0.927	0.220	0.0003
LFEM	–0.642	0.206	0.0010
LINT	0.403	0.205	0.0500

FSOC in Tab. 7). Thus, ageing in a group of flies improved performance in both sexes. An effect of HOUR was short of significance; it would mean that DC1 increased with daytime. In *C. fuscimana*, MMAT had an effect on SDC: the more days a male had maturated, the longer SDC became.

Previous authors noted an influence of maturation time and body size. In *C. amoena*, aged flies tended to copulate faster than young ones, and it was considered exceptional when in a Swiss stock TC increased with male age (BAND, 1995a). In *D. melanogaster*, aged males were seen to have a mating advantage over young ones (LONG *et al.*, 1980) and large males over small ones (PARTRIDGE *et al.*, 1987; PITNICK, 1991). In the present data, maturation time and body size of *C. costata* had too weak an influence to become statistically significant among the other covariates. However, in bivariate linear regression plots, TC1 increased slightly along with

Tab. 9. Independent variables which were used in multiple regression analysis (BMDP2L), with information on range and average. LMAL, LFEM, LINT are dummy variables, used in Tab. 8. HOUR and LUX are used in both species, COND only in *C. fuscimana*; all three apply to both sexes. The other variables were noted separately for females and males; the point before the name stands for either F or M (female or male). With LUX, LOC and ORIG, numbers in parenthesis are arbitrary scores. ML: in most cases, the length of the mesonotum (including the scutellum) of the etherized fly was measured with a binocular microscope in units of 0.1 mm. The remaining flies were measured on the screen at standstill when backplaying the videorecord.

Chymomyza	co-variable	meaning	females Min	Max	mean	males Min	Max	mean
costata	LMAL							
	LFEM							
	LINT							
	HOUR	time at begin of recording	8	17	12.1			
	LUX	light intensity: dim (1) to bright (4)	1	4	2.7			
	.ML	mesonotum length in microscopic units	20	32	28.2	18	33	25.8
	.MAT	days of maturation	1	38	15.0	0	33	10.7
	.SOC	number of flies maturating in a vial	1	12	4.8	1	10	4.3
fuscimana	COND	hours spent in conditioning cage	0	40	12.9	0	40	12.9
	.LOC	stock Gockhausen (1) or Tinizong (2)	1	2	1.3	1	2	1.2
	.ORIG	stock: emergee (1) or adult (2); captured (3); reared from larvae in beech (4) or spruce (5)	1	5	2.5	1	5	2.4
	.MAT	days of maturation	1	54	16.0	1	35	15.8
	.SOC	number of flies maturating in a vial	1	10	2.5	1	9	2.8

both maturation time and body size of male *C. costata*, while DC1 and SDC decreased. In all such plots a large scatter abolished statistical significance, except in the case of TC1, where p=0.048.

The same independent variables as above were used in a discriminant analysis (BMDP7M) to compare two subsets of *C. costata* pairs: those which copulated and those which did not copulate during the 3 hours of videorecording. In the plot of the two groups, no separation became evident. It also appeared that the two subsets did not significantly differ with respect to any of the independent variables.

ZUSAMMENFASSUNG

Das Verhalten von drei in Schweizer Wäldern vorkommenden *Chymomyza*-Arten wurde auf Videoband registriert. Dazu wurden Einzelpaare während etwa 3 Stunden in 4 nebeneinander geordneten Beobachtungskammern aufgenommen. Die Arten unterschieden sich in der Anzahl Kopulationen, in der Zeit, die bis zur ersten Kopulation verstrich (time to first copulation, TC1), in der Dauer der ersten Kopulation (duration of first copulation, DC1) und der Summe aller DC Werte je Paar (SDC). Bei den zwei Arten mit den meisten Paaren wurden innert 3 Stunden bis zu 4 Kopulationen registriert. DC nahm von der ersten bis zur vierten Kopulation ab. Von grösstem Einfluss auf TC1, DC1 und/oder SDC war die Kombination von Partnern aus zwei Stämmen. Gründer des einen stammen aus einem Mittellandwald bei Zürich, des anderen aus einem alpinen Hangwald im Oberhalbstein. Der Nachweis einer beträchtlichen Streuung bei drei Paarungsvariabeln widerlegt eine in neuerer Zeit geäusserte Annahme, das Paarungsverhalten einer Art sei innerhalb ihres «natürlichen Habitats» invariabel (PATERSON, 1993).

ACKNOWLEDGEMENTS

The present study was stimulated by papers of Dr. H. T. BAND. She commented on the manuscript, pointed out relevant papers and helped with the English.

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(received November 14, 1995; accepted March 12, 1996)