Eur. J. Entomol. **93**: 451–459, 1996
ISSN 1210-5759

Effects of interaction between temperature and CO_2 on life-history traits of two *Drosophila* species (Diptera: Drosophilidae)

Josselyne BOULETREAU-MERLE and Daniel SILLANS

Laboratoire de Génétique des Populations, Université Claude Bernard, Lyon I, Bât. 403, 69622 Villeurbanne, France

***Drosophila melanogaster*, *D. simulans*, temperature, carbon dioxide, developmental duration, viability, fecundity**

Abstract. The effects of interaction between continuous exposure to different low CO_2 concentrations (0% and 5%) and temperatures (14 and 25°C) on several life-history traits were studied in the two sibling species *D. melanogaster* and *D. simulans*. The various combinations of the two factors were applied during egg-to-adult development and during adult life.

Developmental duration was increased by CO_2, particularly at low temperature (14°C), and viability reduced. At low temperature, body size was increased, although wing-length variation was reduced by CO_2 in this condition. The fecundity of flies reared at low temperature was reduced due to a decrease in the number of ovarioles while carbon dioxide often improved egg production in both species. CO_2, unlike temperature, did not appear to be a powerful selective factor, but modified the expression of certain traits, being, in some cases, beneficial to the flies.

INTRODUCTION

The two widespread sibling species, *Drosophila melanogaster* and *D. simulans*, are confronted with variations in environmental factors over the greater part of their geographical area, from the Equator to 52 and 45° latitude respectively. Both species, laying eggs and developing on decaying fruits, experience increases in CO_2 concentration due to fermentation during certain stages of their life cycle, as well as changes in temperature due to seasonal variation in temperate regions. The effects of these factors have been well studied separately, but their interaction is less fully documented.

Temperature variation has long been identified as a primordial factor in the development and morphogenesis of insects, in general, and of *Drosophila*, in particular (David & Clavel, 1966, 1967; McKenzie, 1978), and has been identified as a selective factor for genetic variations in natural populations, including seasonal and geographical variations in *Drosophila* (Boulétreau-Merle et al., 1986, 1992).

Various effects of carbon dioxide on insects are also known, and have been reviewed by Nicolas & Sillans (1989). CO_2 induces narcosis and modifies respiration, cardiac activity and behaviour; as a modifier of susceptibility to insecticides, it is used for the disinfestation of fruits and stored seeds (Bailey & Banks, 1980; Whiting et al., 1992; Locatelli & Daolio, 1993). In *Drosophila*, the sigma virus induces sensitivity to short pure CO_2 exposure (L'Héritier, 1948). Continuous exposure to low concentrations (< 20%) during development induces proportional modifications in developmental duration, viability and adult size (Boulétreau et al., 1984). Similar indirect effects have been observed in a lepidopteran reared on its host plant, grown in a high CO_2 concentration (Fajer et al., 1989; Bazzaz, 1990).

Comparative studies have already been performed between the two sibling species: *D. simulans* appears to be more sensitive to short-term exposure to pure CO_2 at high temperature, with longer recovery (McCrady & Clark, 1983), and the more sensitive to continuous exposure (Boulétreau et al., 1984).

This paper presents interaction effects of continuous exposure to 5% CO_2 concentration (CO_2 condition) in comparison with an effectively 0% concentration (normal atmosphere = N condition) at two constant temperatures (14°C and 25°C) during larval and/or adult stages.

MATERIAL AND METHODS

Stocks of *D. melanogaster* and *D. simulans* were recently founded from two asympatric populations near Lyon (France) and mass-bred in the laboratory. Both were insensitive to short exposure to pure CO_2.

For the experiments, normal atmosphere or atmosphere with 5% pure CO_2 was delivered, in plastic boxes (35 x 25 x 13 cm) placed in thermostatic incubators, by a gas-mixing pump (Wösthoff). Two temperatures were used: 25° and 14°C.

Batches of 100 eggs were deposited in vials containing 20 g corn-yeast medium (David & Clavel, 1965), closed by expanded polyurethane allowing gas circulation. Between 2,100 and 3,000 isoparental eggs were used for each of the four rearing conditions (25°C-N; 25°C-CO_2; 14°C-N; 14°C-CO_2), for both species.

Emerging adults were counted, and their sex determined, three times a day at 25°C and once a day at 14°C. Developmental duration was calculated separately for the two sexes. Egg-to-adult viability was estimated as the ratio of number of emerging flies to number of eggs.

For each rearing condition, biometric traits (wing length and live body weight) were measured on fifty 36–48 h old females. The females were then dissected and their number of ovarioles determined.

Fecundity for each of the four rearing-condition groups was studied in each of the same four conditions in adult life, for both species. For each of these 16 combinations, 10 groups of five pairs were formed at emergence and placed in small plastic boxes fitted with air-holes covered by a metal gauze on two facing sides. Nutritive medium, placed in a trough (1.76 cm^2) at the bottom of the box, was renewed every day at 25°C or every two days at 14°C (in view of the difference in egg-laying rates). The eggs laid on this substrate were counted for 25 days at 25°C and for 30 days at 14°C.

Interaction effects between temperature and carbon dioxide conditions were tested by Anova 2.

The coefficient of variation (C.V. given in %) was calculated: $\frac{S.D.}{\bar{x}} \times 100$.

RESULTS

Developmental duration

Developmental duration at 14°C was approximately four times as long as at 25°C (Table 1). The development of *D. simulans* was generally faster than that of *D. melanogaster*, except at 25°C in normal atmosphere (25°C-N). In both species, females emerged before males. The presence of carbon dioxide increased significantly the developmental period in both species (Student's t varying from 13.9 to 20.8 for *D. melanogaster* according to rearing temperature and sex, and from 7.1 to 8.8 for *D. simulans*; $p < 0.0001$; df: 40 to 56). The effect, estimated as percentage of developmental duration in normal atmosphere, was stronger for *D. melanogaster* than for *D. simulans* and stronger at 14°C (14°C-CO_2) than at 25°C (25°C-CO_2). The interaction between the two factors was significant in both species ($F = 459.17$ and $F = 94.43$ respectively: $p < 0.0001$; df: 1). By way of example, Fig. 1 shows this shift towards longer duration, for males of the two species, at 14°C (14°C-N / 14°C-CO_2).

TABLE 1. Variation in developmental duration and viability according to rearing conditions.

		25°C				14°C			
		No. eggs	Viability (%)	Dev. dur. females (days)	Dev. dur. males (days)	No. eggs	Viability (%)	Dev. dur. females (days)	Dev. dur. males (days)
Drosophila melanogaster									
Normal atm.	$\bar{x} \pm$ S.E.	2,100	83.33 ± 0.81	9.11 ± 0.01	9.28 ± 0.01	2,200	73.0 ± 1.51	38.36 ± 0.07	39.73 ± 0.08
	C.V. (%)		4.48	3.74	4.88		9.70	5.05	5.82
5% CO_2	$\bar{x} \pm$ S.E.	2,100	81.38 ± 1.15	9.75 ± 0.01	10.04 ± 0.02	2,300	68.74 ± 1.17	44.60 ± 0.09	46.59 ± 0.11
	C.V. (%)		6.50	4.41	5.61		8.16	5.73	6.43
Variation	$\frac{CO_2 - N}{N}$(%)		−2.34	+6.93	+8.19		−5.84	+16.29	+17.27
Drosophila simulans									
Normal atm.	$\bar{x} \pm$ S.E.	2,800	73.79 ± 1.32	9.14 ± 0.02	9.39 ± 0.02	3,000	61.10 ± 1.05	35.83 ± 0.07	36.87 ± 0.08
	C.V. (%)		9.45	7.23	7.47		9.43	6.61	6.90
5% CO_2	$\bar{x} \pm$ S.E.	2,800	72.04 ± 1.05	9.48 ± 0.02	9.90 ± 0.03	2,800	58.50 ± 2.10	38.24 ± 0.06	39.41 ± 0.06
	C.V. (%)		7.72	7.29	9.34		18.96	4.48	4.65
Variation	$\frac{CO_2 - N}{N}$(%)		−2.37	+3.73	+5.40		−4.25	+6.72	+6.88

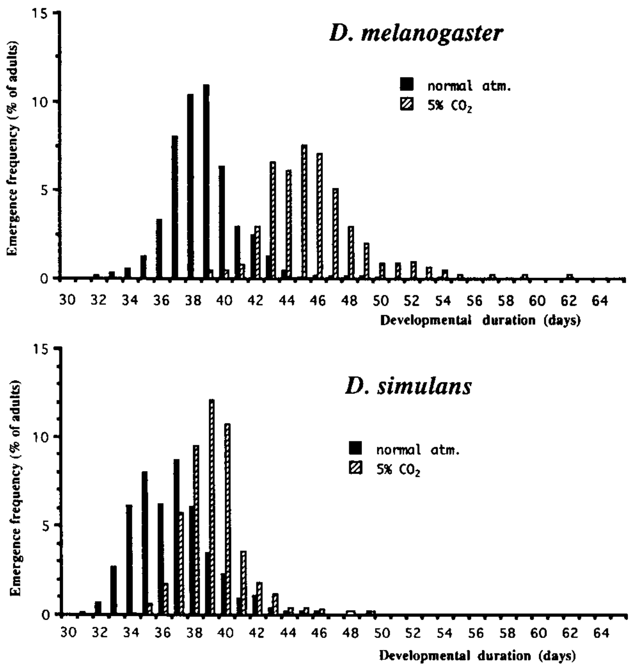

Fig. 1. Effects of CO_2 on the developmental duration of male *Drosophila* reared at 14°C: frequency of emergence as percentage of adults emerging.

Viability

Egg-to-adult viability was relatively low for both species in spite of the recent foundation of the strains, and particularly low for *D. simulans*. Carbon dioxide and especially, low temperature both reduced the number of emerging adults (Table 1). Association of the two factors (14°C-CO_2) induced a severe decrease: 14.59% for *D. melanogaster* and 15.29% for *D. simulans*.

Biometry

Numerical data related to female biometrical traits are presented in Table 2. Development at low temperature induced a significant increase in wing length in both species ($F = 553.2$; $p < 0.0001$ for *D. melanogaster*; and $F = 58.2$; $p < 0.0001$ for *D. simulans*). It decreased severely the number of ovarioles ($F = 322$ and $F = 256$ respectively; $p < 0.0001$). It also induced a decrease in body weight in *D. melanogaster* ($F = 19.9$; $p < 0.0001$), but had no significant effect on *D. simulans*, due to the variability of the trait at 25°C.

The presence of carbon dioxide had no significant effect on wing length at 25°C in either species, but did moderate significantly the increase induced by low temperature development ($t = 4.7$; $p < 0.0001$ for females of *D. melanogaster* and $t = 2.3$; $p < 0.02$ for *D. simulans*). CO_2 had no clear effect on body weight and the significant, inverse, variations observed at 25°C for the two species were, probably, an artefactual consequence of age: the heavier females having been measured about 12 hours later than the other group of females and, thus, having more mature eggs in the ovarioles. CO_2 had no effect on the number of ovarioles in *D. simulans*, but slightly and significantly decreased ovariole number in *D. melanogaster* ($t = 3.63$; $p < 0.0004$; df: 113).

The combination of the two factors (14°C-CO_2) affected significantly wing length ($F = 24.1$; $p < 0.0001$ for *D. melanogaster* and $F = 5.7$; $p < 0.018$ for *D. simulans*), body weight ($F = 14.8$; $p < 0.0002$ and $F = 8.1$; $p < 0.0049$, respectively) and, for *D. melanogaster* only, the number of ovarioles ($F = 5.7$; $p < 0.017$ and $F = 1.3$; $p < 0.259$, respectively).

TABLE 2. Interaction effects of temperature and carbon dioxide during development on biometrical traits of *Drosophila* females ($\bar{x} \pm 1$ S.E.; second line: C.V.).

	Rearing conditions	Wing length (1/100 mm)	Body weight (1/100 mg)	Ovariole number
Drosophila melanogaster	25°-Normal	267.22 ± 0.65	155.20 ± 1.67	49.69 ± 1.67
		1.71	7.60	10.40
	25°C-CO_2	269.15 ± 0.84	146.20 ± 1.36	49.69 ± 0.60
		2.62	7.86	10.12
	14°-Normal	295.94 ± 1.09	141.83 ± 2.08	39.88 ± 0.67
		2.61	10.38	11.80
	14°C-CO_2	288.52 ± 0.90	145.22 ± 1.38	36.86 ± 0.52
		2.52	7.65	11.35
Drosophila simulans	25°-Normal	234.94 ± 0.64	127.08 ± 1.27	39.26 ± 0.48
		1.93	7.07	8.65
	25°C-CO_2	235.48 ± 0.57	136.60 ± 1.53	39.62 ± 0.49
		1.70	7.91	8.73
	14°-Normal	251.02 ± 0.82	131.06 ± 1.94	32.42 ± 0.49
		2.30	10.49	10.65
	14°C-CO_2	239.90 ± 0.86	130.06 ± 2.43	31.74 ± 0.37
		2.53	13.23	8.22

Fecundity

Fecundity is influenced by developmental conditions that modify larval reserves and number of ovarioles and by adult living conditions which determine vitellogenesis rate, egg production and egg laying rate.

However, temperature acts so powerfully on fecundity expression that fecundity will be studied separately as expressed at 25°C and at 14°C. The curves of daily fecundity in normal atmosphere, during the 25 or 30 days of experiment, according to expression temperature are presented in Figure 2. The global fecundity values observed over 25 days under 25°C or over 30 days under 14°C are presented in Table 3.

EXPRESSION AT 25°C. 25°C is a favourable temperature for egg production in *Drosophila*, in which daily fecundity is more or less correlated with the number of ovarioles, in both species. As can be seen from the above, development at low temperature decreased the number of ovarioles in both species and the presence of CO_2 enhanced this effect in *D. melanogaster* only.

TABLE 3. Variation of mean global fecundity according to rearing and adult living conditions; $\bar{x} \pm 1$ S.E.; second line: C.V.

	Rearing conditions	Adult life conditions			
		25°-Normal	25°C-CO_2	14°-Normal	14°C-CO_2
Drosophila melanogaster	25°-Normal	827.04 ± 14.88 5.69	813.04 ± 16.77 6.52	118.28 ± 9.49 25.36	80.92 ± 10.58 41.35
	25°C-CO_2	762.32 ± 14.40 5.97	798.48 ± 19.99 7.92	180.96 ± 11.73 20.50	144.74 ± 15.15 33.10
	14°-Normal	694.61 ± 14.63 6.66	621.20 ± 23.71 12.07	188.68 ± 10.06 16.86	188.56 ± 8.23 13.80
	14°C-CO_2	490.34 ± 27.12 17.49	479.31 ± 32.00 21.11	181.14 ± 6.67 11.64	151.75 ± 11.5 23.96
Drosophila simulans	25°-Normal	520.39 ± 16.16 9.82	571.57 ± 11.39 6.30	142.88 ± 6.48 14.34	120.74 ± 13.90 36.41
	25°C-CO_2	505.32 ± 6.15 3.85	535.67 ± 19.4 11.45	137.40 ± 7.01 16.13	136.79 ± 7.12 16.45
	14°-Normal	307.12 ± 14.27 14.69	375.96 ± 19.44 16.35	65.07 ± 8.11 39.43	53.73 ± 5.32 31.33
	14°C-CO_2	291.09 ± 16.52 17.95	331.78 ± 10.78 10.28	75.65 ± 7.55 31.54	51.42 ± 11.81 72.64

Thus, mean mated female egg-production was about 40 eggs per day for *D. melanogaster* (Fig. 2A) and 30 eggs for *simulans* (Fig. 2B) when reared at 25°C, and fell to 30 and 25 eggs per day for *D. melanogaster*, reared at 14°C without and with CO_2 respectively (Fig. 2A) and to about 20 eggs per day for both groups of *D. simulans* reared at 14°C (Fig. 2B). These daily fecundity rates are not the optimum production of the two species, which would be obtained with isolated pairs. The day to day variability in the fecundity of *D. melanogaster* was a consequence of the supply of nutritive medium which was renewed every day, but manufactured every two days: the flies preferred to lay on fresh medium.

The presence of CO_2 in the atmosphere during adult life decreased slightly the fecundity of *D. melanogaster* (Table 3), except for flies reared under 25°C-CO_2 where there was an increase which, however, was non significant ($p < 0.16$). The decrease observed for flies reared under 14°C-N was the only significant variation ($t = 2.63$; $p < 0.01$; df: 18). However, CO_2 increased fecundity in *D. simulans* at 25°C whatever the rearing conditions. The variations here were all significant ($p < 0.01$) except for the flies reared under 25°C-CO_2.

EXPRESSION AT 14°C. At 14°C, egg production was reduced drastically ($F = 3964$ for *D. melanogaster* and $F = 2911$ for *D. simulans*; $p < 0.0001$; df: 1). Egg-laying began later than at 25°C and fecundity never exceeded 10 eggs per day (Fig. 2C, D). In spite of this

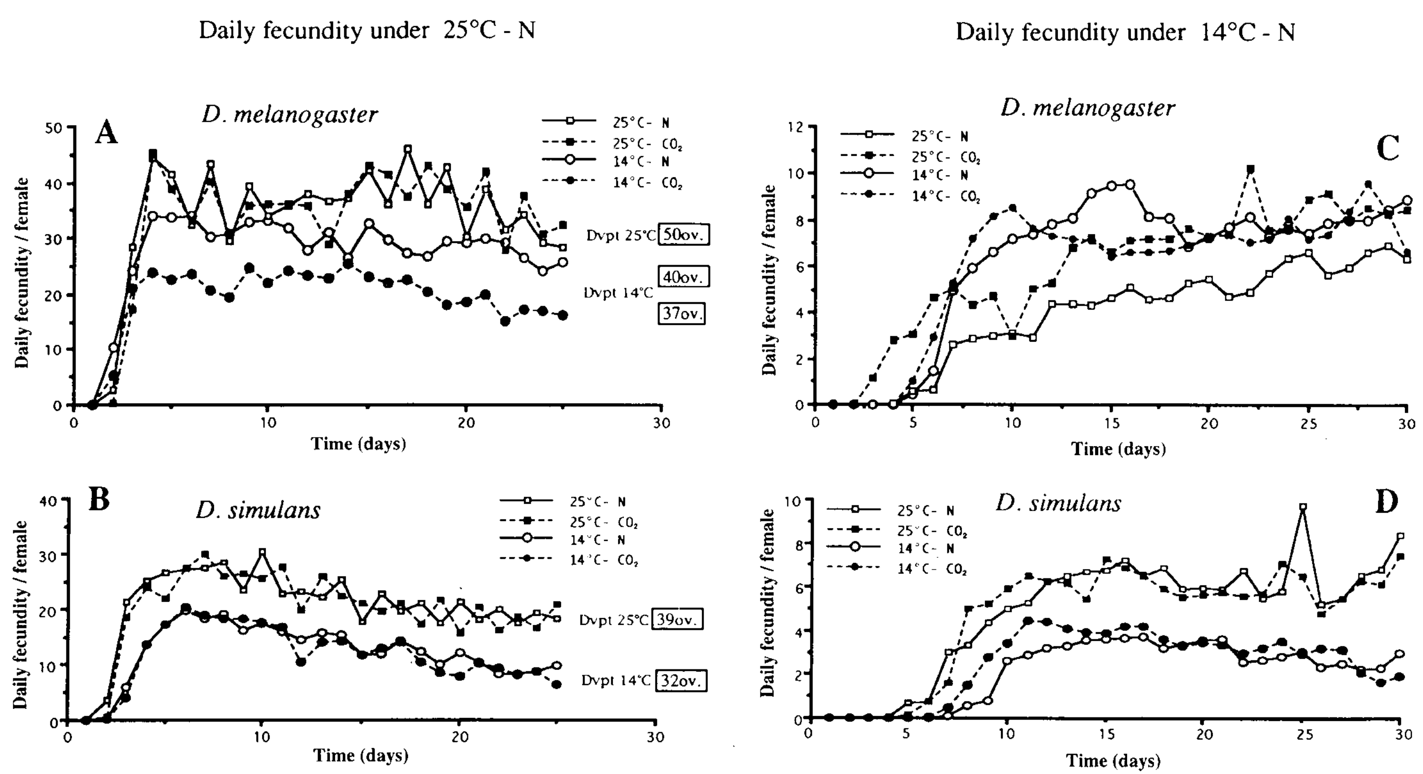

Fig. 2. Influence of rearing conditions on the subsequent daily fecundity of *D. melanogaster* and *D. simulans* observed at 25°C and 14°C, in normal atmosphere. Flies reared under 25°C: □■; flies reared under 14°C: ○●. Flies reared under 5% CO_2: closed symbols and dotted lines. Flies reared under normal atmosphere: open symbols, full lines. Dvpt: Development under 25 or 14°C. In frame: the number of ovarioles (ov.) observed in each rearing conditions.

low level, a consequence of a slowing down of metabolism, some differences could be discerned.

Females of *D. melanogaster* reared at 25°C, despite their higher number of ovarioles, exhibited lower fecundity than flies reared at 14°C ($t = 4.8$; $p < 0.0001$; df: 78), the lowest rate being for flies reared in normal atmosphere (25°C-N) (Fig. 2C).

The fecundity of *D. simulans* at 14°C reflected the number of ovarioles and, as at 25°C, flies reared at 25°C showed at higher fecundity than those reared at 14°C ($t = 14.85$; $p < 0.0001$; df: 78).

At 14°C, the presence of carbon dioxide during adult life always decreased fecundity in both species, whatever the rearing condition (Table 3). However, the variation estimated by t-test for flies from a given rearing condition, was significant only for *D. melanogaster* reared under 25°C-N ($p < 0.01$) or 14°C-CO_2 ($p < 0.04$) or for *D. simulans* reared at 14°C-CO_2 ($p < 0.003$). The influence of CO_2 estimated by ANOVA and F-Test for all groups taken together, was significant ($F = 5.99$ for *D. melanogaster* and $F = 6.57$ for *D. simulans*; $p < 0.01$). The interaction effect between temperature and CO_2 during adult life, estimated by the same ANOVA was non-significant in *D. melanogaster* but significant in *D. simulans* ($F = 28.78$; $p < 0.0001$).

The two factors acting during development influenced the fecundity of the adults by modifying the number of ovarioles, and it was difficult to identify other possible effects of developmental conditions on fecundity, independently of this ovariole-number effect. However, in spite of a serendipitously identical number of ovarioles, a difference occurred between the groups of *D. melanogaster* reared at 25°C, with or without CO_2: the flies reared with CO_2 (25°C-CO_2) produced a higher number of eggs at 14°C (162.85 ± 10.21, as against 99.6 ± 8.14 for 25°C-N; $t = 4.85$; $p < 0.0001$; df: 38).

DISCUSSION AND CONCLUSION

The influence of temperature variation and of carbon dioxide acting separately on the various traits have been studied on one (David & Clavel, 1966, 1967, 1969; Sillans & Fouillet, 1990) or both (Boulétreau et al., 1984) species. The association of the two factors, with the use of a low temperature, brought out new aspects, showing that temperature and carbon dioxide do not have equally strong effects on the various life-history characteristics of *Drosophila*.

The choice of the two temperatures and two concentrations of CO_2 may be a cause of this difference, since 14°C and 25°C are relatively extreme in the range of viable developmental temperatures: from 12°C to 31°C for *D. melanogaster* and 11°C to 29°C for *D. simulans*; furthermore, 5% CO_2 is a low concentration at the threshold of significant effectiveness in previous studies. However, the various experimental conditions used here are such as may actually be experienced by flies in natural populations and the effects observed may correspond to the reality of the field.

Temperature variations are already known to modify developmental duration and egg-to-adult viability. Generally, the present results are in agreement with the values already determined for *D. melanogaster* (David & Clavel, 1966) although viability at 25°C was lower than the 100% viability observed by Boulétreau et al. (1984) and that generally observed in the laboratory. It is possible that the higher hygrometric level occurring in our closed boxes induced this difference.

The modifications in biometric traits induced by temperature variation have long been known in *D. melanogaster* (David & Clavel, 1967) and our results show that *D. simulans* presents similar variations. It is to be noted that the increase in body size induced by low temperature was associated with a reduction in the number of ovarioles, important for the subsequent fecundity of the flies.

The increase in developmental duration induced by carbon dioxide in both species has already been shown at 25°C, this increase being correlated to the CO_2 concentration in the atmosphere (0 to 20%) (Boulétreau et al., 1984). The present results show that this effect is enhanced by low temperature (14°C) particularly for *D. melanogaster*. The presence of carbon dioxide had a slight negative effect on viability, whatever the developmental temperature or the species.

The reducing effect of CO_2 on biometric traits was weak and significant only at low temperature, suggesting that an interaction occurs between the two factors; it is interesting to note that flies with longer development emerged with reduced size which is not the usual process for insects. The decrease in ovariole number at 14°C was significant for the subsequent fecundity of the *D. melanogaster* females.

It is known that fecundity depends on the temperature at which adults are living (David & Clavel, 1969) and, to a lesser extent, on the number of ovarioles, varying in the present case with rearing conditions. The difference in effectiveness of the two factors acting during adult life was clear with regard to this trait. Temperature induces a difference in general metabolic rate and, consequently, in egg production, while CO_2 modulates this production only, generally lowering it. However, CO_2 had a favourable effect on fecundity in two cases: in *D. melanogaster*, CO_2 applied during development at 25° enhanced subsequent fecundity of females at 14°C and in *D. simulans* all the females from the four rearing conditions maintained at 25°C-CO_2 had higher fecundity than aliquot females observed at 25°C in normal atmosphere. One hypothetical explanation of this effect could be a slight inhibition of oviposition control. 25°C is higher than the optimal temperature determined for *D. simulans* fecundity. Under these suboptimal conditions, each female controlling egg deposition and egg production centrally may reduce its fecundity rate. The low level of CO_2 may act as light anaesthetic, decreasing this control. The same control exists in *D. melanogaster*, where such an effect was found only in the case of flies reared under 25°-CO_2, but the optimal temperature for this species is close to 25°C. The effect was observed for this species under 28°C-CO_2 (unpublished).

The two species reacted similarly to the environmental conditions. However *D. simulans*, showing generally smaller variations, appeared to be less influenced by CO_2 during its rearing phase: the increase in developmental duration was weaker and biometric traits appeared unaffected.

Its appears that carbon dioxide is not an important factor for adaptive modifications occurring in natural populations, particularly in comparison with temperature. However, the increase in developmental duration might be favourable for overwintering populations such as *D. melanogaster*, adult flies emerging later in spring from refuges. Apparently, *D. simulans* cannot overwinter in the Lyon region, and recolonises the area each year from southern regions (unpublished). Even so, its avoidance of human constructions (McKenzie & Parsons, 1972; Fuyama & Watada, 1981; Rouault & David, 1982; Yamamoto et al., 1985) and its lower longevity under low temperature (unpublished) are probably more important than its shorter developmental duration in the incapacity for overwintering at this latitude, as is observed in *D. melanogaster*.

REFERENCES

Bailey S.W. & Banks H.J. 1980: A review of recent studies of the effects of controlled atmospheres on stored product pests. In Shybal J. (ed.): *Controlled Atmosphere Storage of Grains*. Elsevier, New York, pp. 101–118.

Bazzaz F.A. 1990: The response of natural ecosystems to the rising global CO_2 levels. *Annu. Rev. Ecol. Syst.* **21**: 167–196.

Boulétreau M., Fouillet P. & Sillans D. 1984: Differential sensitivity of Drosophila melanogaster and Drosophila simulans to chronic exposure to carbon dioxide during development. *Experientia* **40**: 566–567.

Boulétreau-Merle J., Terrier O. & Fouillet P. 1986: Developmental temperature as a selective factor in balancing polymorphisms in D. melanogaster populations. *J. Therm. Biol.* **11**: 143–149.

Boulétreau-Merle J., Fouillet P. & Terrier O. 1992: Clinal and seasonal variation in initial retention capacity of virgin D. melanogaster females as strategy for fitness. *Evol. Ecol.* **6**: 223–242.

David J. & Clavel M.-F. 1965: Interaction entre le génotype et le milieu d'élevage; conséquences sur les caractéristiques du développement de la Drosophile. *Bull. Biol. Fr. Belg.* **49**: 369–378.

David J. & Clavel M.-F. 1966: Essai de définition d'une température optimale pour le développement de la Drosophile. *C. R. Acad. Sci. Paris* **262**: 2159–2162.

David J. & Clavel M.-F. 1967: Influence de la température subie au cours du développement sur divers caractères biométriques des adultes de Drosophila melanogaster Meigen. *J. Insect Physiol.* **13**: 717–729.

David J. & Clavel M.-F. 1969: Influence de la température sur le nombre, le pourcentage d'éclosion et la taille des oeufs pondus par Drosophila melanogaster. *Ann. Soc. Entomol. Fr.* **5**: 161–177.

Fajer E.D., Bowers M.D. & Bazzaz F.A. 1989: The effects of enriched carbon dioxide atmospheres on plant-insect herbivore interactions. *Science* **243**: 1198–1200.

Fuyama Y. & Watada M. 1981: The microdistribution of Drosophila melanogaster and Drosophila simulans: a survey in the Bonin Islands. *Zool. Mag.* **90**: 62–68.

L'Héritier P. 1948: Sensitivity to CO_2 in Drosophila. A review. *Heredity* **2**: 325–348.

Locatelli D.P. & Daolio E. 1993: Effectiveness of carbon dioxide under reduced pressure against some insects infesting packaged rice. *J. Stored Prod. Res.* **29**: 81–87.

McCrady W.B. & Clark M.A. 1983: Variation in response to CO_2 in Drosophila melanogaster and D. simulans. *J. Hered.* **74**: 171–174.

McKenzie J.A. 1978: The effect of developmental temperature on population flexibility in Drosophila melanogaster and D. simulans. *Aust. J. Zool.* **26**: 105–112.

McKenzie J.A. & Parsons P.A. 1972: Alcohol tolerance; an ecological parameter in the relative success of Drosophila melanogaster and Drosophila simulans. *Oecologia* **10**: 373–388.

Nicolas G. & Sillans D. 1989: Immediate and latent effects of carbon dioxide on insects. *Annu. Rev. Entomol.* **34**: 97–116.

Rouault J. & David J.R. 1982: Evolutionary biology of Drosophila melanogaster and D. simulans; a behavioral divergence in microhabitat selection. *Acta Oecol.* **3**: 331–338.

Sillans D. & Fouillet P. 1990: Réponse de Drosophila melanogaster à des atmosphères enrichies de façon permanentes en CO_2. *Bull. Soc. Ecophysiol.* **15**: 115–122.

Whiting D.C., Van den Heuvel J. & Foster S.P. 1992: Potential of low oxygen/moderate carbon dioxide atmospheres for postharvest disinfestation of New Zealand apples. *N. Z. J. Crop Hortic. Sci.* **20**: 217–222.

Yamamoto A.H., Fuyama Y. & Watada M. 1985: Habitat selection of two sibling species, Drosophila melanogaster and D. simulans: a further survey in the Bonin Islands. *Zool. Sci.* **2**: 265–270.