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Survival and reproduction of small blow flies (*Calliphora vicina*; Diptera: Calliphoridae) produced in severely overcrowded short-day larval cultures

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Abstract. In diapause-destined larval cultures of the blow fly, *Calliphora vicina*, competition through severe overcrowding forces down body size and causes the smaller individuals to escape from the diapause "programme" to form miniature puparia. Among the diapausing cohort, smaller larvae contain a similar proportion of fat to larger larvae, and a similar rate of fat metabolism over the first 7 weeks in diapause. However, these smaller diapausing larvae are much less cold tolerant than larger individuals. Of the miniature larvae that side-step the diapause programme to form puparia, even the smallest of them are capable of development to the adult, and these small-sized adults are capable of depositing "full-sized" eggs (although fewer of them) which hatch to produce "full-sized" and viable larvae. These data suggest that the smallest individuals, by avoiding the diapause programme, may gain a selective advantage by completing another autumnal generation instead of entering diapause and failing to survive because of a reduced cold tolerance.

INTRODUCTION

When adult females of the blow fly, *Calliphora vicina*, are exposed to long daylength they produce a further generation of continuously-developing or non-diapausing progeny which promptly pupariate, but when they are exposed to autumnal short days they give rise to larvae that may enter a prolonged diapause, commencing shortly after the end of feeding (Vinogradova & Zinovjeva, 1972; Saunders et al., 1986). The maternal critical daylength for larval diapause induction was found to be about 14.5L : 9.5D for a population of *C. vicina* isolated in south-eastern Scotland (Saunders, 1987).

Diapause incidence amongst autumnal or diapause-programmed populations of *C. vicina* may be reduced by several environmental factors, including elevated temperatures acting upon the mother (Saunders, 1987; McWatters & Saunders, 1998) or upon the larvae she produces (Vaz Nunes & Saunders, 1989). Also, in grossly overcrowded larval populations produced by short-day mothers, the smaller individuals may "side-step" their diapause programme to form miniature puparia, whereas larger individuals in the same cohort proceed to larval diapause, as expected (Saunders, 1997). Although diapausing larvae and non-diapausing pupae, of all sizes, lay down the same *proportions* of fat, smaller diapausing larvae appear to have a shorter (shallower) diapause that may be a reflection of a higher metabolic rate (Saunders, 1997). Consequently, it was suggested that smaller larvae would be less well equipped for an extended period of over-wintering, and might therefore gain some selective advantage by following the non-diapause pathway (pupariation and subsequent emergence). With this "bet-hedging" strategy (Cohen, 1966; Philippi & Seger, 1989), smaller larvae "opt" for the chance to produce further progeny before the winter finally sets in, whereas larger larvae with sufficient reserves enter diapause and delay reproduction until the following season.

To lend credence to this "bet-hedging" hypothesis, it needs to be shown (1) that small diapausing larvae are at a disadvantage when compared to their full-sized siblings, and (2) that the smaller individuals that side-step the diapause programme to form puparia are capable of surviving to reproduction. This paper addresses these questions by determining rates of fat consumption and resistence to freezing of "large" and "small" diapausing larvae, and by determining survival and reproduction rates for different size classes of those individuals that avoided the diapause programme.

MATERIAL AND METHODS

Culture methods

Stock blow flies (*Calliphora vicina* Robineau-Desvoidy) were maintained at 25°C under continuous light, since their isolation in Musselburgh (51°N), near Edinburgh, in the late summer of 1984. Adult flies were provided with sugar and water ad libitum, and with meat (beef muscle) from about day 4 post eclosion. Larvae hatching from eggs laid on this meat were reared to maturity in cultures supplemented by an agar, dried milk and yeast medium (Saunders, 1987), and allowed to disperse into dry sawdust to form puparia. Under these conditions all larvae showed non-diapause development.

Experimental cultures of flies were maintained in small gauze-covered cages (26 × 21 × 19 cm), similarly provisioned with sugar, water and meat. These cages were enclosed in wooden cabinets (37 × 36 × 30 cm) housed in a walk-in constant temperature room at 22°C. Each cabinet was fitted with a 4 W fluorescent striplight controlled by a timeswitch to give daily photoperiods of either 12L : 12D (a "short" day) or 18L : 6D (a

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"long" day). Eggs collected on days 9 to 12 of adult life were allowed to hatch and the hatchlings established as larval cultures held in the dark (DD) at 11°C. Under these conditions, larvae from long-day parents underwent prompt (non-diapause) development to puparia, whereas a variable proportion of those from short-day parents entered diapause after wandering from their food into the sawdust (Saunders et al., 1986). Since diapausing larvae were morphologically indistinguishable from nondiapause-destined larvae, all larvae that failed to pupariate after 30 days in culture were considered to be in diapause, as in earlier papers (Saunders, 1987, 1997).

Experimental design

Newly hatched larvae from short-day parents were transferred to standard "hamburgers" made from 50 g of minced beef muscle, at densities of either 50 larvae/50 g of meat, or 350 larvae/50 g meat, and then placed at 11°C and constant darkness for development. The lower densities represented uncrowded cultures in which larvae had ample food; the higher densities gave rise to severely overcrowded cultures, leading to a marked reduction in body size (Saunders & Bee, 1995). Larvae were allowed to wander from these cultures naturally, either when they had finished feeding, or when they left an overcrowded culture through lack of food or, perhaps, tainting of the medium.

Puparia were collected daily from the sawdust and weighed individually on a Sartorius microbalance. Those individuals that entered diapause were similarly weighed, on day 30 post-oviposition. In the severely overcrowded cultures larval mortality was high (see below), often when the larvae were very small. Dead larvae were not recorded as such, because they could not be determined as either diapausing or nondiapausing individuals.

Diapausing larvae were sorted into two size classes: "small" larvae of less than 30 mg fresh weight, and "large" larvae of 40 to 70 mg. Intermediate weight larvae (30 to 40 mg) were excluded from the experiment in order to produce two distinctly separate size classes. Amongst those individuals that side-stepped the diapause programme, puparia were sorted into 6 size classes (10–20, 20–30, 30–40, 40–50, 50–60 and > 60 mg) and placed, as groups, in cages at 19°C, 12L : 12D for further development.

Fat determination

At the start of diapause (day 30 of the cultures) and at weekly intervals thereafter, samples of 10 "small" and 10 "large" diapausing larvae were weighed, dried overnight in an oven at 40–50°C, and re-weighed. They were then extracted with chloroform-methanol (2 : 1 vv) to remove soluble lipids, dried and weighed again. The extraction procedure was repeated, usually at least twice, until a constant fat-free dry weight was obtained. Fat contents for individual larvae were then expressed as a percentage of their dry weight.

Exposure to cold

At the onset of diapause (day 30) groups of 10 "small" or 10 "large" larvae were placed in flat-bottomed glass tubes immersed in a water bath enclosed in a low-temperature incubator, and maintained at –8°C using a commercial antifreeze (see Saunders & Hayward, 1998, for details). At daily intervals up to 12 days, tubes of "small" and "large" larvae were removed from the water bath and their contents tipped into petri dishes at 25°C. Transferring diapausing larvae of *C. vicina* to this temperature results in rapid diapause termination (Saunders et al., 1986). The initial recovery of the larvae from cold exposure was recorded after 45 to 60 min at 25°C and was determined by movement in response to touching. Formation of a normal tanned and barrel-shaped puparium (Žďárek, 1988) was then recorded, followed by eclosion which was deemed to have been successful when the fly had completely emerged from its puparium and was not deformed.

Development and reproduction of small individuals that side-stepped the diapause programme

Each of the 6 size groups of puparia that avoided the diapause programme was scored for successful eclosion (complete emergence of non-crippled flies) and for sex ratio. Emerging flies were then maintained at 19°C, 12L : 12D, and supplied with meat to obtain eggs and subsequent larval progeny. For each size group, the lengths of 30 randomly selected eggs were measured and, after the resulting larvae had left their meat, the weights of 30 mature larvae were recorded.

RESULTS

Effects of larval crowding on body size and survival

Using first instar larvae produced by flies exposed to short days, cultures comprising 50 larvae/50 g of meat ("normal" cultures), or of 350 larvae/50 g of meat ("overcrowded" cultures) were established. The less crowded (normal) cultures produced a high yield of mature larvae or pupae (average = 50 per culture), but the overcrowded cultures suffered an estimated mortality of about 60% (559 survivors from an initial, combined inoculum of 1,400 first instar larvae). As in earlier investigations overcrowding led to a marked reduction in body size (Saunders & Bee, 1995), and also to an increased proportion of the smaller larvae avoiding the diapause "programme" (Saunders, 1997). In the present experiments, the proportion of individuals that formed puparia rather than entering diapause rose from about 30% in "normal" cultures to 43% in the overcrowded cultures.

Fat contents of large and small diapausing larvae

Fat contents of 10 "large" (40 to 70 mg) and 10 "small" (< 30 mg) diapausing larvae were determined at the arbitrary start of diapause (30 days post-oviposition) and at weekly intervals thereafter for 7 weeks. Fig. 1 shows that both groups of larvae contained approximately the same proportions of fat (expressed as a percentage of dry weight), with no obvious changes occurring over the 7 week period.

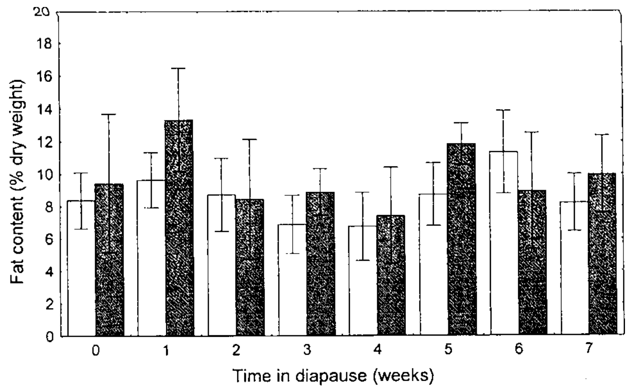

Fig. 1. Fat contents of "large" (40–70 mg fresh weight; open columns) and "small" (< 30 mg; hatched columns) diapausing larvae of *Calliphora vicina*, showing that these two size classes of larvae have similar fat contents across 7 weeks of diapause development. Bars represent 2 × SEM.

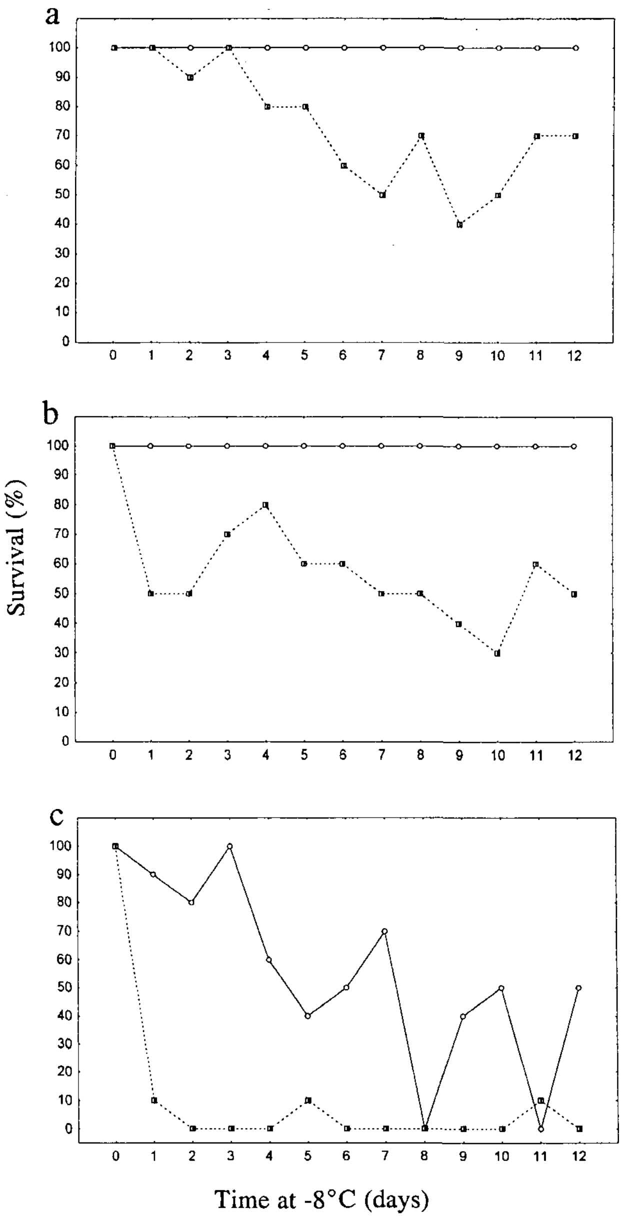

Fig. 2. Survival of "large" (40–70 mg fresh weight; O——O) and "small" (< 30 mg; ◨-----◨) diapausing larvae of *C. vicina* exposed to a temperature of –8°C for 1 to 12 days before transfer to 25°C. a – initial recovery; b – subsequent pupariation; c – survival to eclosion of non-crippled adult fly.

Cold tolerance of large and small diapausing larvae

Fig. 2 shows survival rates of "large" and "small" diapausing larvae exposed to –8°C for 1 to 12 days. After transfer to 25°C, all of the large larvae survived to form puparia whereas 34/120 (28.3%) of the small larvae failed to show an initial recovery from chilling (Fig. 2a) and 55/120 (45.8%) of them failed to form a puparium (Fig. 2b). As for successful eclosion (Fig. 2c), 57/120 (47.5%) of the large larvae failed to become non-crippled adults, whereas almost all (117/120; 97.5%) of the small individuals died before this stage. The data for the survival of "full-sized" diapausing larvae at –8°C are similar to those described earlier for the Edinburgh strain of *C. vicina* (Saunders & Hayward, 1998). It was concluded that small (< 30 mg) larvae are much less tolerant of chilling to –8°C than their "full-sized" (40–70 mg) siblings.

Survival and reproductive success of the small flies that escaped from the diapause programme

Table 1 presents data on survival and reproduction of nondiapausing individuals according to their size (pupal weight) category. Emergence rates were high (> 96%, non-crippled flies) in all categories, and the sex ratio was between 39 and 64% of females. Although there was an overall eight-fold range in pupal weights (10.3 to 82.8 mg), flies in all size groups laid eggs measuring between 1.35 and 1.56 mm in length (overall mean 1.46 mm). Since these eggs subsequently hatched, they were judged to be both "full-sized" and viable. Mean weights of mature larvae produced by the six size groups varied from 57 to 71 mg, differences probably being the result of the degree of crowding within the available food. It was concluded, therefore, that even the smallest pupae gave rise to viable flies that were capable of laying full-sized eggs which, in turn, hatched to normal larvae. The *number* of eggs laid by small-sized flies was not recorded, but earlier results (Saunders & Bee, 1995) demonstrated a positive correlation between fecundity and body size: small flies, therefore, lay full-sized and viable eggs but fewer of them.

DISCUSSION

Gross overcrowding among the larvae produced by short-day (diapause programmed) females of the blow

TABLE 1. Eclosion success of different-sized blow flies (*Calliphora vicina*) from overcrowded cultures, and the sizes of the eggs and larvae they produce. Emerging flies were kept at 19°C under short daylength (12L : 12D); the larvae they produced were then maintained at 11°C in darkness. Mean values ± 2 SE.

Pupal size category (mg)	Number of pupae	Number emerging (%)	Females (%)	Egg size (mm) N = 30	Larval size (mg) N = 30
10–20	32	31 (96.9)	45.2	1.42 ± 0.024	68.73 ± 2.773
20–30	89	88 (98.9)	47.7	1.44 ± 0.013	61.68 ± 3.254
30–40	36	36 (100)	38.9	1.49 ± 0.017	71.43 ± 3.713
40–50	28	28 (100)	64.3	1.50 ± 0.022	61.65 ± 2.572
50–60	31	31 (100)	48.4	1.46 ± 0.020	68.71 ± 2.118
> 60	51	49 (96.1)	46.9	1.47 ± 0.017	56.99 ± 2.653

fly, *Calliphora vicina,* leads to reduced body size and a decrease in the incidence of larval diapause (Saunders & Bee, 1995; Saunders, 1997). When analysed according to body weight, the data show clearly that heavier short-day larvae tend to proceed to diapause, whereas lighter larvae may opt for the nondiapause pathway, forming miniature puparia. Saunders (1997) suggested that the smallest individuals may gain a selective advantage by following the nondiapause pathway because they have insufficient reserves for winter survival, whereas larger larvae with ample reserves promptly enter diapause. If this "bet-hedging" hypothesis is to be regarded as realistic, it must be demonstrated that smaller diapausing larvae are at a disadvantage when it comes to overwintering survival, whereas the smallest individuals that opt for the nondiapause pathway are capable of surviving to reproduction.

Although diapausing larvae of all sizes lay down a similar proportion of fat, smaller diapausing larvae show a shorter diapause than larger larvae, suggesting that the former may have a higher metabolic rate and therefore be less well equipped for an extended period of overwintering (Saunders, 1997). Data presented in this paper confirm that small and large diapausing larvae have comparable fat contents, but fail to indicate any differences in rates of fat consumption. Tolerance of a period of chilling (–8°C), however, was shown to be considerably greater for larger larvae. This suggests that – although a temperature of –8°C is probably an extreme condition for overwintering larvae in a sub-terranean environment – the cold tolerance of small diapausing larvae is much less than that of their normal sized siblings. Small size for diapausing larvae of *C. vicina* is, therefore, almost certainly a disadvantage.

On the other hand, the smallest individuals that opt for the nondiapause pathway (pupariation) seem to suffer little disadvantage. Even the smallest pupae hatch successfully (> 96% in all size categories) and the small adults that emerge are capable of laying "full sized" and viable eggs, although fewer of them (Saunders & Bee, 1995). Furthermore, larvae hatching from these eggs develop to "normal" size. This ability of very small flies to reproduce successfully recalls the behaviour of the related blow fly, *Lucilia cuprina*, under similar conditions of severe overcrowding (Nicholson, 1957).

Available data suggest that such a density-dependent effect on diapause avoidance could occur under natural conditions. *C. vicina* is a multivoltine species with a short generation time. The maternal critical daylength, comprising actual daylength plus the two periods of civil twilight which together may constitute the effective photoperiod (Beck, 1980), would be passed in early September, and the female flies would probably become fully diapause programmed about 10 days later (Saunders, 1987). If the weather remained warm it is likely that many larvae would avoid diapause because of the effects of elevated temperature on the adults (Saunders et al., 1986) or the larvae (Vaz Nunes & Saunders, 1989). However, even if it became cooler, overcrowding of larvae within their limited resource (animal carrion) would provide an additional impetus towards nondiapause development. *C. vicina* is also able to develop, albeit rather slowly, at quite low temperature: egg development may proceed at 3.5°C, larval development down to 4°C, and progression to the adult at 5°C (Davies & Ratcliffe, 1994). Therefore, given a relatively mild autumn, a further generation of flies might be expected whose progeny would, in turn, become dormant and enter the overwintering population. Results presented in this paper, therefore, suggest that avoidance of diapause in overcrowded populations of *C. vicina* may indeed be an important natural phenomenon, offering some selective advantage to small autumnal breeders.

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