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Pleiotropic Effects of the *'ecdysoneless-1'* Mutation of *Drosophila melanogaster*

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Summary. At the restrictive temperature (29° C), ring glands of the temperature-sensitive mutant *'ecdysoneless-1'* (*ecd-1*) of *Drosophila* secrete ecdysteroids in vitro at a low rate compared to wild-type. The ring gland, brain and also the larval salivary glands of non-pupariating *ecd-1* larvae are smaller than wild-type and this lack of tissue development may be a major factor determining the low rate of ecdysteroid secretion by the ring gland. A heat pulse in larval development causes cell death in imaginal disc tissues and the adults emerging after such treatment display a variety of morphological abnormalities. These effects suggest that the *ecd-1* mutation is a temperature-sensitive cell lethal and has more general effects on cell and tissue growth than has been supposed. Furthermore, we interpret the autonomous ovarian defects of *ecd-1* females at the restrictive temperature in terms of non-lethal effects on the follicle epithelial cells. The diverse phenotypic effects of this mutation may represent autonomous pleiotropy and we urge caution in interpreting the defects of *ecd-1* flies only in terms of ecdysteroid deficiency.

Introduction

Moulting and metamorphosis, fundamental events in the insect life cycle, are initiated by 20-hydroxyecdysone and related compounds (ecdysteroids) (review: Richards 1981). An understanding of the way in which ecdysteroids control these events, and interact with other hormones, juvenile hormone in particular, is of major relevance to the study of gene regulation and differentiation. This may be achieved, at least in part, by the isolation and analysis of ecdysteroid mutants of *Drosophila*. The mutation *ecdysoneless-1 (l(3)ecdysone-1^{ts}*; homozygotes are abbreviated throughout to *ecd-1)*, a temperature-sensitive mutation in *Drosophila melanogaster*, has been suggested to represent a primary defect in the pathway of ecdysone synthesis or its regulation (Garen et al. 1977). Larvae homozygous for this mutation have greatly reduced ecdysteroid titres at the restrictive temperature and have been used for studies on the role of ecdysone during larval development (Marsh and Wright 1980; Kraminsky et al. 1980).

Ecdysteroids have been identified in adult insects (review: Richards 1981) and are present in low, but apparantly significant amounts in adult *Drosophila* (Hodgetts et al. 1977; Handler 1982). The role of ecdysteroids in *Drosophila* adults, particularly with regard to ovarian development, so far remains obscure (review: Bownes 1982). The analysis of ecdysteroid mutants is one approach to this problem and there have been attempts to interpret the sterility of adult *ecd-1* females (Garen et al. 1977) in terms of a reduction in ecdysteroid titres (Garen et al. 1977; Audit-Lamour and Busson 1981). However, *ecd-1* remains largely uncharacterised as to its primary defect and to conclude that the sterility and defective ovaries of *ecd-1* flies (Audit-Lamour and Busson 1981) result from hormone deficiency may be premature. From a series of experiments on *ecd-1* homozygotes we have found that this mutation has effects very similar to temperature-sensitive cell-lethal mutations and we suggest that the reduction of ecdysteroid titres in *ecd-1* larvae and adults (Garen et al. 1977) is a secondary consequence of cell death and an inhibition of tissue growth. In view of this, and the effects of the *ecd-1* mutation on the growth of larval tissue, we urge caution in interpreting any functional deficiency of *ecd-1* flies at the restrictive temperature in terms of ecdysteroid deficiency.

Materials and Methods

Animal Culture. The Oregon-R (OrR, wild type) and *l(3)ecdysone-1^{ts} st ca/l(3)ecdysone-1^{ts} st ca* strains of *Drosophila melanogaster* were cultured at 18° C on a standard cornmeal, agar, yeast and sugar medium (see Lindsley and Grell 1968, for details of other mutations). For experiments with 3rd instar *ecd-1* larvae at the restrictive temperature, eggs were collected over a 4–6 h period at 18° C and the larvae transferred to 29° C approximately 7 days later (Garen et al. 1977). Larvae collected using this protocol are specifically referred to as 'non-pupariating *ecd-1* larvae'. For the 20-hydroxyecdysone feeding experiments, non-pupariating larvae, 3 days after the 18–29° C temperature shift, were fed 20-hydroxyecdysone as a 1 mg/ml solution in a yeast paste containing 5% ethanol. For some experiments with pupae and prepupae, staged rather than timed animals were used: the staging system was that of Bainbridge and Bownes (1981). The staging system of King (1970) was used for scoring ovarian development.

Tissue Culture and Transplantation. Ring glands and ovaries for the transplantation experiments were dissected in

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Grace's medium (as supplied by Flow laboratories) or Ringers (Chan and Gehring 1971), respectively, and transplanted into larval or adult hosts using the method of Ephrussi and Beadle (1936).

Yolk-protein synthesis by 'fat bodies' (abdominal body wall preparations) and ovaries was followed by culturing the tissues for 2 h at 29° C in 20 µl of Ringers containing 50 µCi S^{35} methionine (specific activity 800–1,100 Ci/mmole, Amersham). Labelled proteins in the tissue or culture medium were analysed with polyacrylamide gel electrophoresis either directly or after antibody precipitation with an anti-yolk antibody (Isaac and Bownes 1982). Proteins present in the haemolymph and other tissues of adult flies were analysed by electrophoresis followed by protein staining with Coomassie Blue (Bownes and Hames 1977).

Brain-ring gland preparations for the analysis of ecdysteroid output were dissected from *ecd-1* and OrR larvae and cultured as described by Redfern (1983).

The non-vital dye Trypan blue was used to stain for cell death in imaginal discs (Dale and Bownes 1981).

Ecdysteroid Radioimmunoassay (RIA). Radioimmunoassay, performed as described by Redfern (1983), was used to estimate ecdysteroid levels in crude 70% methanolic extracts of whole insects and the rate of ecdysteroid secretion by brain-ring gland preparations cultured in vitro. For these latter experiments, the culture medium only was assayed (Redfern 1983). Changes in ecdysteroid levels in whole insects during prepupal and pupal development were investigated by transferring *ecd-1* and OrR white prepupae to 29° C and assaying 4–8 samples, 2–6 insects per sample, at 6-h intervals. Insects were weighed and extracted in 70% methanol containing 10^{-3} M phenylthiourea, as described in Bainbridge et al. (1982). Ecdysteroid levels in adult females were estimated by RIA of 4–5 groups, 5 females per group, of *ecd-1* and OrR females respectively. In these experiments, the 70% methanolic extracts were partially purified by chloroform partition (Lafont et al. 1982) in order to remove chloroform-soluble material which we have since found to cross react or interfere with the assay. Methanolic extracts were reduced under nitrogen to 100–200 µl and partitioned against 100 µl of chloroform. The chloroform phase was backwashed 3 times with 200 µl of distilled water and the aqueous phases combined and freeze-dried for RIA. The extraction efficiency of exogenous H^3 ecdysone (NEN, 80 Ci/mmole) was 94.4% using this procedure. The ecdysone antiserum used in these experiments was the HORN I2 (16 wks) ecdysone-22-hemisuccinate antiserum, a generous gift of Dr. J.D. O'Connor. Authentic samples of 20-hydroxyecdysone (Sigma) or ecdysone (Simes, purified by HPLC) were used as Standards.

Results

Ecdysteroid Synthesis by ecd-1 Ring Glands

The increase in ecdysteroid titre occurring just before puparium formation in wild type *Drosophila* (Hodgetts et al. 1977) is prevented in *ecd-1* larvae transferred to 29° C as late 2nd or early 3rd instar larvae (Garen et al. 1977). A failure to synthesise hormone or to regulate the activity of catabolising enzymes are two factors which could equally give rise to this *ecd-1* phenotype. To distinguish between these alternatives, 'brain-ring gland preparations' from non-pupariating *ecd-1* larvae (see Materials and Methods), dissected at the time when control OrR larvae of the same age were pupariating, were cultured in vitro at 29° C and the amounts of ecdysteroid secreted into the culture medium were assayed by RIA. Brain-ring glands from OrR larvae, dissected one-day after the 18° C to 29° C temperature shift, were cultured and assayed as controls. The results (Fig. 1) show that *ecd-1* brain-ring glands have a greatly reduced rate of ecdysteroid secretion compared to the OrR controls. From this we conclude that the *ecd-1* larval phenotype results from a failure of the ring glands to produce sufficient hormone. This conclusion is supported by the results of ring gland transplantation experiments in which the ring glands from wandering 3rd instar OrR larvae were implanted into non-pupariating *ecd-1* larvae: 42% (5/12) of the *ecd-1* hosts formed tanned 'prepupae' within 2 days.

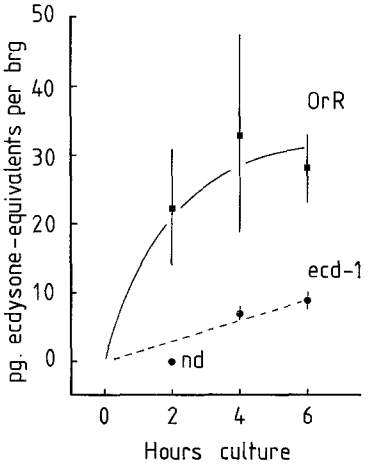

Fig. 1. Ecdysteroid secretion by brain-ring gland preparations (brg) from nonpupariating *ecd-1* larvae (●) and OrR wild-type larvae (■) cultured in vitro for 2, 4 or 6 h at 29° C. Ecdysteroids present in the culture medium after incubation were assayed by RIA. nd = not detectable. Error bars: ±2SEM

It is possible that the failure of *ecd-1* ring glands to produce sufficient ecdysteroid at the restrictive temperature is due to a defect in the biosynthetic pathway or its regulation by prothoracicotropic hormone from the brain. However, the brain, ring gland and also the larval salivary glands from non-pupariating *ecd-1* larvae were smaller than in OrR larvae of the same age (Fig. 2), and it is likely that the failure of *ecd-1* ring glands to produce sufficient hormone is a non-specific result of an inhibition of tissue growth rather than a specific defect in ecdysteroid production or its regulation.

'Puparium' formation may be induced in non-pupariating *ecd-1* larvae by feeding with 20-hydroxyecdysone (Garen et al. 1977) and in two repeats of such an experiment, 28% and 37% of *ecd-1* larvae formed tanned 'prepupae' under our culture conditions. However, we prefer to call the animals which result from 20-hydroxyecdysone feeding, or from the implantation of a wild-type ring gland, "pseudoprepupae" because although the larval skin was tanned and the anterior spiracles everted, these animals failed to contract into the characteristic 'barrel' shape of true prepupae (Bodenstein 1950) and did not develop further. Non-pupariating *ecd-1* larvae may therefore have other defects preventing normal development in response to hormone.

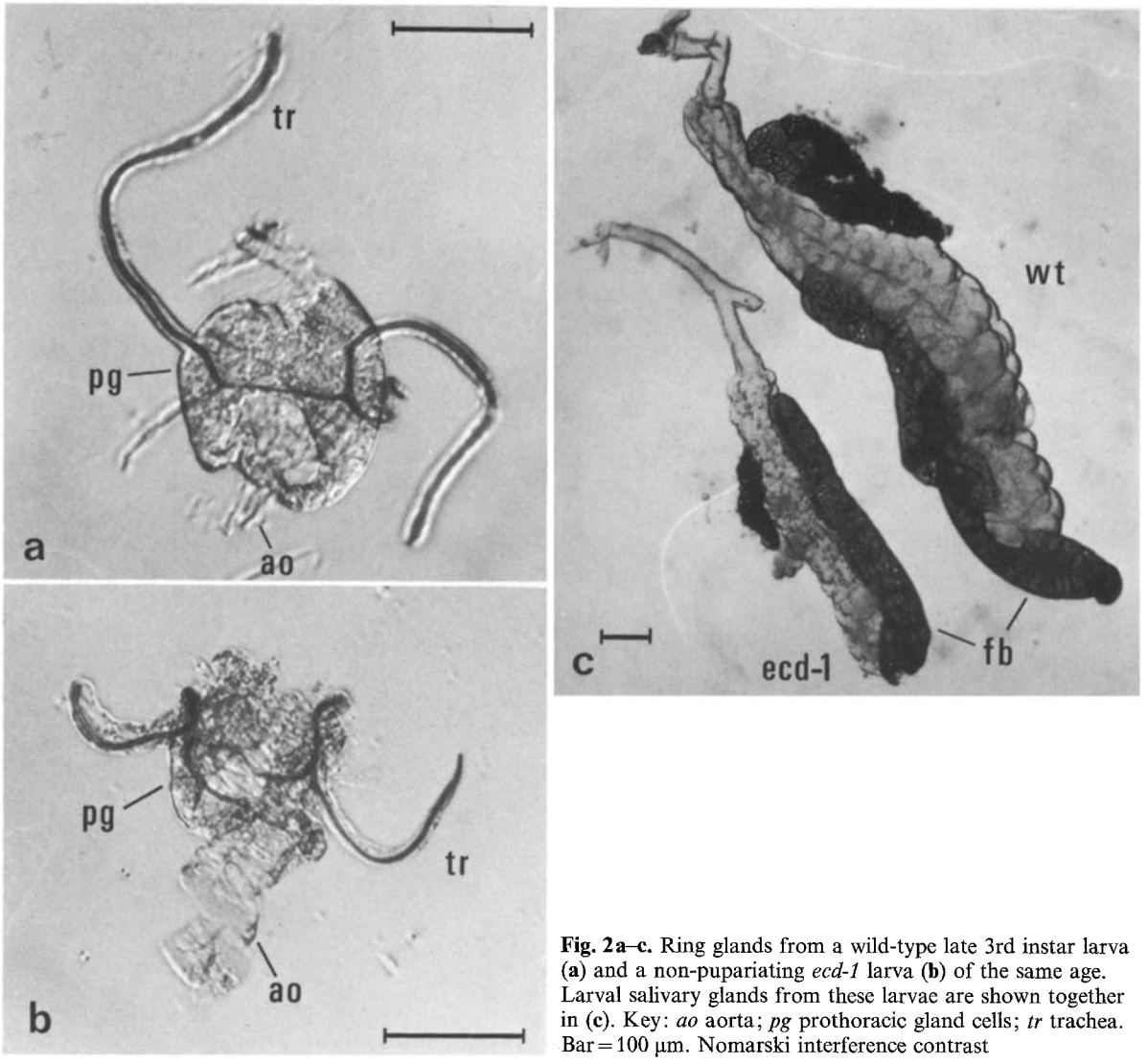

Fig. 2a–c. Ring glands from a wild-type late 3rd instar larva (**a**) and a non-pupariating *ecd-1* larva (**b**) of the same age. Larval salivary glands from these larvae are shown together in (**c**). Key: *ao* aorta; *pg* prothoracic gland cells; *tr* trachea. Bar = 100 μm. Nomarski interference contrast

The Effects of a Temperature Shift During Pupal Development: Developmental Defects and Ecdysteroid Titres

The restrictive temperature does not prevent pupal and subsequent pharate adult development in *ecd-1* animals transferred to 29° C at puparium formation, but these animals fail to eclose. By transferring animals to 29° C at various times after puparium formation, we found that adults only eclosed if they had been transferred 45 h or more after puparium formation. In this we concur with the recently-reported findings of Audit-Lamour and Busson (1981). We have repeated these temperature-shift experiments using *ecd-1* pupae staged according to the system described by Bainbridge and Bownes (1981): animals transferred to 29° C at stage P6 (12/12) or P7 (22/22) eclosed successfully whereas 58% (7/12) of those transferred at stage P5(ii) and all those transferred at stage P5(i) (0/6) or earlier (0/38) failed to eclose. Head eversion markes the transition from stages P4(ii) to P5(i) (Bainbridge and Bownes 1981) and, using the approximate developmental times given by Bainbridge and Bownes (1981), the temperature-sensitive period therefore ends approximately 20 h (18° C) after this event.

The pharate *ecd-1* adults which failed to eclose after a temperature shift in prepupal or early pupal development had a variety of morphological defects: macrochaetes were reduced in size, missing or misshapen, abdominal bristles were reduced in size and number and midline fusion of abdominal segments appeared abnormal. All major structures (eyes, legs etc.) were present but the genitalia were often poorly differentiated. Emerging adults from *ecd-1* pupae transferred to 29° C after the temperature-sensitive period appeared normal in all respects.

During normal development, the ecdysteroid titre decreases after puparium formation (Hodgetts et al. 1977), reaching a low level at stages P4 to P5(i) before rising again to reach a peak during stages P7 to P13 (Bainbridge et al. 1982). In view of the effects associated with development at 29° C from the white prepupal stage, we were interested in determining whether or not the increase in ecdysteroid titre that should occur during this period was prevented at 29° C. Samples of *ecd-1* and control OrR animals, maintained at 29° C from puparium formation, were extracted and assayed using RIA. The results (Fig. 3) show that the titre of cross-reacting material in these crude extracts increases in both strains of flies during this period of develop-

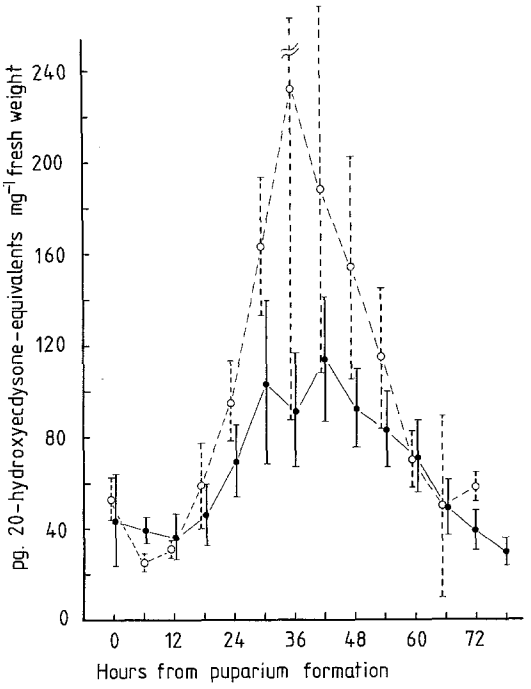

Fig. 3. Ecdysteroid titres during metamorphosis of OrR (o, ...) and *ecd-1* (•, ——) flies: animals were transferred to 29° C at puparium formation and sacrificed for RIA at 6 h intervals thereafter. Error bars: ±Standard deviation

ment. Although the peak titre is lower in the *ecd-1* than OrR animals, we are reluctant to attach much importance to this in view of the high variance of the data. We tentatively suggest that the defects associated with development from puparium formation at 29° C do not result from hormone deficit, and that the production of ecdysteroids and cross-reacting material, as measured in our RIA, is not prevented at 29° C during this period. We should point out that RIA on unpurified extracts gives relatively crude estimates of total ecdysteroid levels. For this reason, we cannot conclude that the titre of *biologically-active* ecdysteroid is unaffected at the restrictive temperature.

The Effects of a Heat Pulse During Development

The bristle and macrochaete defects associated with a transfer to 29° C during prepupal and early pupal development suggested that the *ecd-1* mutation may cause cell death. To study this further, larvae from *ecd-1* eggs collected over a 48 h period at 18° C were subjected to a two-day heat pulse at various times during development (expressed in Tables 1 and 2 as days after egg deposition) and the emerging adults examined for morphological defects. Heat-pulsed animals had a variety of morphological defects (Table 1) and the frequency of these abnormalities reached a maximum when the heat pulse was given in mid-larval development (Table 2), at 5–7 days of age. At this stage, eye and head deformations, similar to those found in flies homozygous for the *tuh* mutation (Bournias-Vardiabasis and Bownes 1978), and homeotic transformations of antenna to leg (Fig. 4), predominated (Table 1). Halteres and genitalia (Fig. 4) were affected by a heat pulse earlier in larval development. Abdominal deformations, in particular poor bristle and pigment differentiation and failure of midline

Table 2. The frequency of abnormal *ecd-1* adults after a 2 day heat pulse at various times during development

Age[a] at shift up (days)	No. examined	% normal	% abnormal	% failed to eclose
0–4	134	97	3	0
3–5	137	35	55	10
4–6	145	37	61	2
5–7	249	6	91	3
7–9	815	79	17.5	3.5
9–11	401	16	12	72
10–12	438	11	5	84
12–14	281	11	1	88

[a] Cultures at ages up to 9–11 days contained larval stages, 10–12 day cultures contained predominantly late 3rd instar larvae but with some prepupae and 12–14 day cultures were predominantly pupae but still with some 3rd instar larvae. All ages refer to the time (days) after egg deposition

Table 1. The frequency of abnormalities in *ecd-1* adults after a 2 day heat (29° C) pulse at various times during development

Defect	Frequency/Age at shift up (days)							
	0–4	3–5	4–6	5–7	7–9	9–11	10–12	12–14
Halteres absent or misshapen		0.24	0.09	0.003		0.04		
Halteres duplicated		0.24	0.01	0.01				
Wing deformed		0.01	0.04				0.04	
Leg missing			0.03					
Leg deformed	1.0	0.01	0.02		0.01		0.04	1.0
Antenna Duplicated			0.02					
Eye or head deformed		0.13	0.16	0.67	0.83	0.04	0.04	
Genitalia deformed[a]		0.36	0.23	0.003	0.006	0.02		
Antenna to leg homeosis			0.29	0.31[b]	0.12[b]	0.14	0.04	
Abdomen deformed			0.09	0.003	0.025	0.76	0.83	
Thorax defective			0.01		0.01			
Total defective flies examined	4	75	80	227	144	49	24	1
Total defects	4	86	90	309	161	49	24	1

[a] Deformations of genitalia were usually a failure to evert correctly

[b] 95.6% of flies with antenna to leg transformation after a heat pulse at 5–7 and 7–9 days old, also had eye defects (Fig. 4)

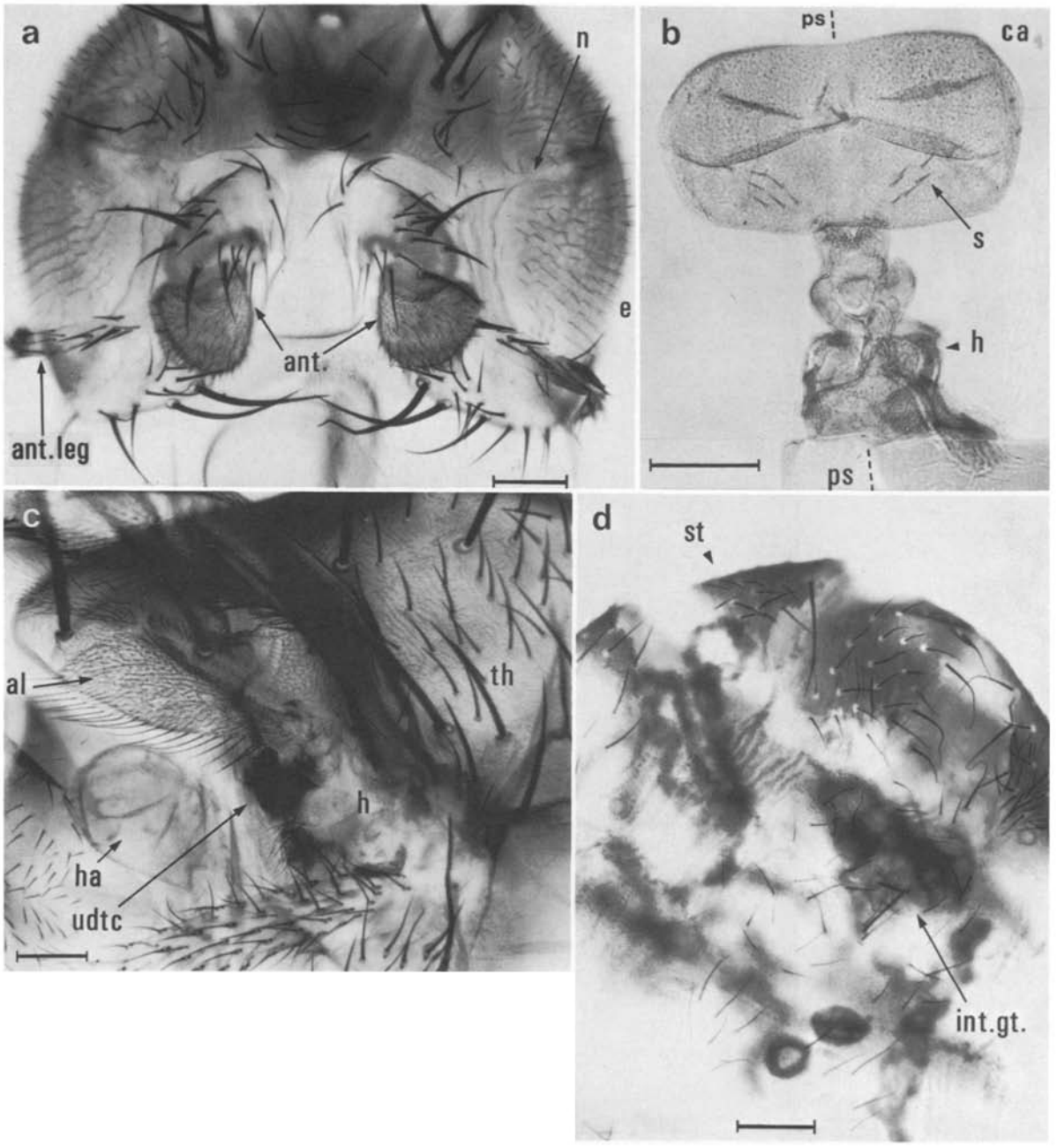

Fig. 4a–d. Morphological deformations in *ecd-1* adults after a heat pulse during larval development. **a** Head, showing homeotic transformation of antenna to leg and 'nicking' in the anterior margin of the eye; **b** mirror-image duplication of a haltere; **c** lateral aspect of thorax showing a patch of undifferentiated, tanned cuticle in the wing hinge region; **d** genital abnormality – the genital disc tissue in this case is lying inside the abdomen. Key: *al* alula; *ant* antenna; *ant.leg* antenna to leg transformation; *ca* capitellum; *e* eye; *int. gt* internal genital disc tissue; *h* hinge; *ha* haltere; *n* nick in anterior margin of eye; *ps* plane of mirror-image symmetry; *s* sensillae; *st* sternite; *th* thorax; *udtc* undifferentiated, tanned cuticle. Bar = 100 μm

fusion at the 2nd and 3rd abdominal segments, resulted from a heat pulse during prepupal and pupal development. Heat pulses in mid to late-larval life lengthened the period of development by as much as 6 days in the case of larvae heat pulsed when 9–12 days old.

Abnormalities in imaginal disc derivatives, resulting from a heat pulse during development, are characteristic of temperature-sensitive cell-lethal mutations in *Drosophila* and may result from localised cell death (e.g. Arking 1975). Imaginal discs from *ecd-1* larvae, transferred in the 2nd or 3rd instar and kept at 29° C for two days, were dissected and stained for cell death with Trypan Blue. Some of these imaginal discs were abnormal in shape and the wing and haltere discs, in particular, had regions with dead cells. Cell death was also seen in the hinge region of wing and haltere discs from non-pupariating 3rd instar larvae that had been maintained at 29° C for 4 days. Wing, leg, haltere and eye-antennal discs from these larvae differentiated very poorly when they were implanted into wild-type 3rd instar larvae and allowed to metamorphose along with the host: normal morphological features were frequently lacking and imaginal cuticle was thin and patchy. Although cell death

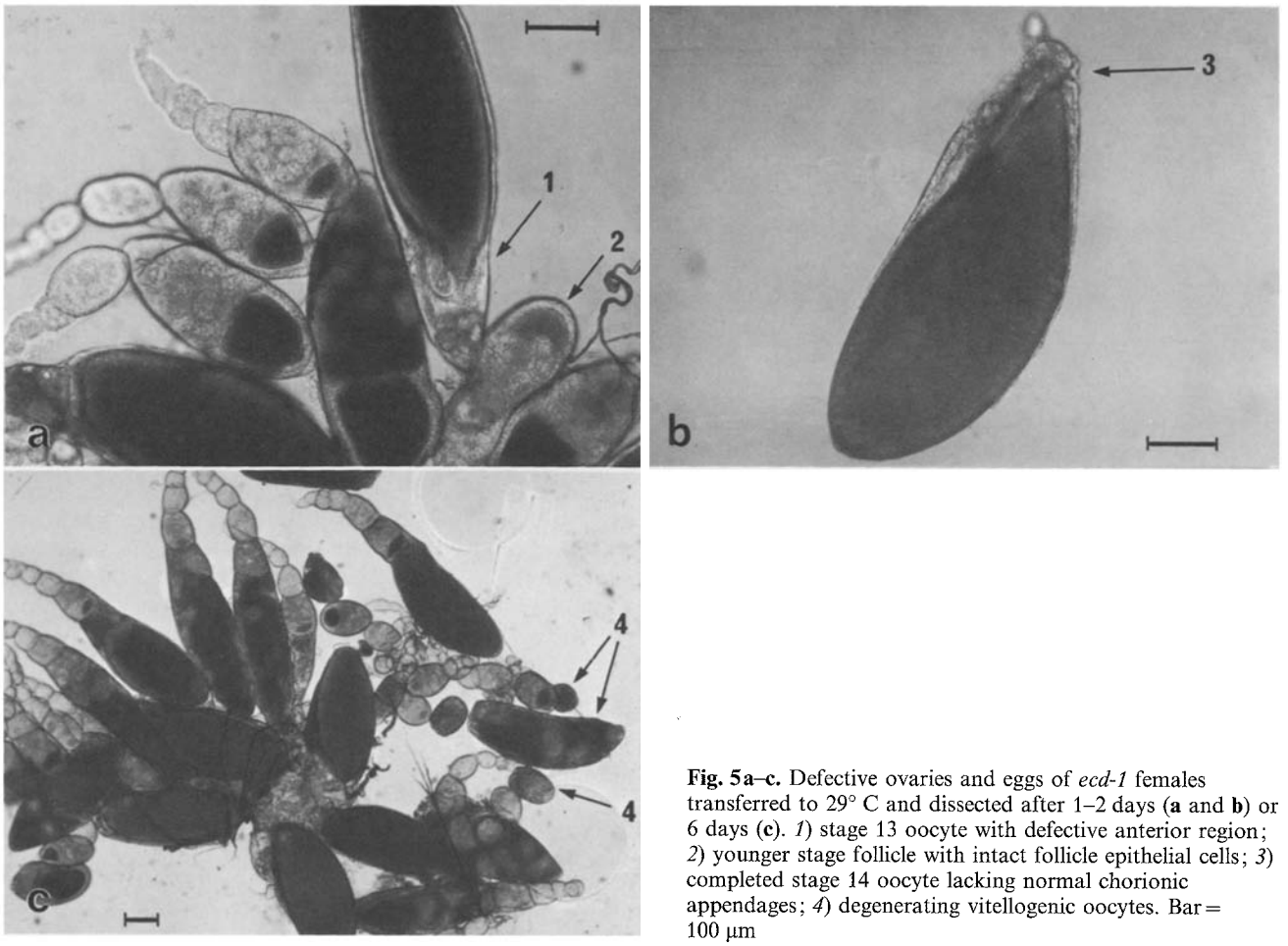

Fig. 5a–c. Defective ovaries and eggs of *ecd-1* females transferred to 29° C and dissected after 1–2 days (**a** and **b**) or 6 days (**c**). *1*) stage 13 oocyte with defective anterior region; *2*) younger stage follicle with intact follicle epithelial cells; *3*) completed stage 14 oocyte lacking normal chorionic appendages; *4*) degenerating vitellogenic oocytes. Bar = 100 μm

was observed in the hinge region of wing discs, the frequency of gross, morphological wing defects in emerging adults was low (Table 1) presumably because the discs regenerated the missing cells (French et al. 1976). However, in addition to the morphological defects scored in Table 1, small patches of undifferentiated, tanned cuticle were present at the wing base of heat-pulsed flies (Fig. 4). The frequency of these patches was not noted, but they presumably relate to cell death in the hinge region of the imaginal disc.

Morphological Defects During Oogenesis at 29° C

Transferring adult *ecd-1* females to 29° C at eclosion or as mature females results in a steady diminution of egg laying and the production of flaccid, infertile eggs with defective chorionic appendages (Audit-Lamour and Busson 1981). In order to look at the changes in ovarian morphology occurring at 29° C, *ecd-1* females were transferred to 29° C at eclosion and the ovaries were dissected and examined at one-day intervals. After a day at 29° C, vitellogenic follicles of all stages were present although stage 14 oocytes had abnormal chorionic appendages (Fig. 5). After 2 days, all the late vitellogenic stages had defective chorionic appendages and earlier vitellogenic stages were degenerating. This pattern remained similar for a least 6 days at 29° C. The ovaries of *ecd-1* females transferred to 29° C 45 h after puparium formation and examined 1–6 days after eclosion contained only previtellogenic stages. It would seem, as Audit-Lamour and Busson (1981) have suggested, that the restrictive temperature prevents the normal development of follicles beyond stage 8 while disrupting chorionogenesis in later vitellogenic stages. The chorion is secreted by the follicle epithelial cells (King 1970) but we have found no evidence of preferential death of these cells in *ecd-1* at the restrictive temperature.

Yolk-Protein Synthesis and the Ability of ecd-1 Hosts to Support the Development of Wild-Type Ovaries

The failure of previtellogenic follicles to develop further does not result from a lack of yolk proteins in the haemolymph: *ecd-1* females transferred from 18° C to 29° C at 45 h after puparium formation and analysed 2–11 days after eclosion had yolk protein in the haemolymph (Fig. 6). The amounts of yolk protein present in these females were broadly similar to wild-type females. Furthermore, both the 'fat bodies' and ovaries from these females, sacrificed 2–5 days after eclosion, still synthesised yolk proteins (Fig. 6), albeit at a reduced rate compared to wild-type.

Yolk-protein synthesis has been suggested to be controlled by 20-hydroxyecdysone in *Drosophila* (Jowett and Postlethwait 1980) and we felt that it was important to confirm that adult *ecd-1* females have reduced ecdysteroid titres at 29° C (Garen et al. 1977). Our results agree closely with Garen et al. (1977): mean (±S.D.) RIA values for flies transferred to 29° C at eclosion and analysed 4 days

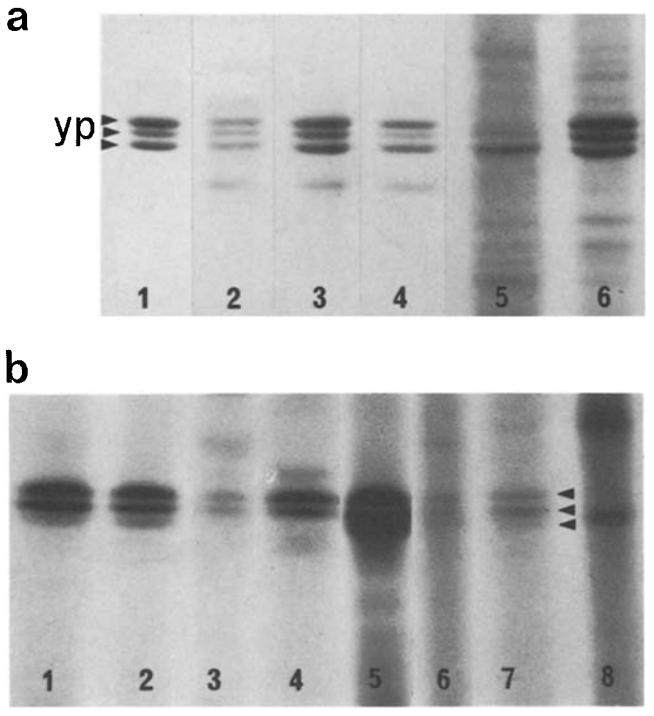

Fig. 6a, b. Yolk-protein synthesis in *ecd-1* and OrR females transferred to 29° C 45 h after puparium formation at 18° C. **a** Protein patterns (stained with Coomassie Blue) in adult *ecd-1* females. Tracks 1–3: haemolymph from ecd-1 females 11 days (track 1, 4 females), 2 days (track 2, 3 females) and 5 days (track 3, 1 female) after eclosion at 29° C. Track 4: haemolymph from 2 OrR females (unknown age). Tracks 5 and 6: proteins from *ecd-1* ovaries (track 5, 3 pairs) and OrR ovaries (track 6, 1 ovary), 5 days after eclosion at 29° C. *yp* yolk polypeptides. **b** Analysis of labelled yolk proteins present in the cells and culture medium of 3 pairs of ovaries or 3 fat bodies after culture in vitro. Tissues were dissected from *ecd-1* or OrR females, 5 days after eclosion at 29° C, and cultured in vitro with s^{35} methionine for 2 h at 29° C. Yolk proteins were then precipitated with an anti-yolk antibody, electrophoresed and fluorographed. Tracks 1–4 are culture medium samples: 1) OrR fat bodies; 2) OrR ovaries; 3) *ecd-1* fat bodies; 4) *ecd-1* ovaries. 'Cells' samples are in tracks 5–8; 5) OrR fat bodies; 6) OrR ovaries; 7) *ecd-1* fat bodies; 8) *ecd-1* ovaries. Yolk polypeptides are arrowed in track 7

later were 1.37 ± 0.16 and 8.92 ± 1.18 pg-ecdysone-equivalents per mg fresh weight for *ecd-1* and OrR females, respectively.

By transplanting larval *ecd-1* ovaries into wild-type hosts and transferring the hosts to 29° C after metamorphosis and maturation at the permissive temperature, Garen et al. (1977) have shown that the sterility of *ecd-1* ovaries is autonomous at 29° C. Wild-type ovaries transplanted into *ecd-1* hosts also ceased egg laying at 29° C in similar experiments (Garen et al. 1977) and this may indicate that *ecd-1* females are unable to support or promote the development of a wild-type ovary or that *ecd-1* females have additional defects of the distal genital tract at the restrictive temperature. To see whether or not the "internal milieux" of *ecd-1* females will allow the development and maturation of wild-type ovaries at the restrictive temperature, we transplanted OrR ovaries, dissected within 2 h of eclosion, into 3-day old female *ecd-1* hosts which had been transferred to 29° C 2 days after eclosion, and examined the implants and host ovaries after 5–6 days at 29° C. The results, together with results from the control experiments in which *ecd-1* ovaries were transplanted into OrR and *ecd-1* hosts, and OrR ovaries were transplanted into OrR hosts using similar experimental conditions, are summarised in Table 3. We conclude that OrR ovaries develop to the same extent whether in *ecd-1* or OrR hosts. *ecd-1* ovaries develop abnormally in a wild-type host and the abnormal ovarian development of *ecd-1* females at 29° C is probably due only to an autonomous ovarian defect.

Discussion

The *ecd-1* mutation clearly has pleiotropic effects, causing morphological abnormalities in response to a heat pulse as well as preventing puparium formation and the increase in ecdysteroid titre (Garen et al. 1977) during late-larval development. The effects of *ecd-1* on morphological development are very similar to those produced by late-larval lethal mutations (Shearn et al. 1971) and temperature-sensitive cell-lethal mutations at the restrictive temperature (Arking 1975; Simpson and Schneiderman 1975; Russell et al. 1977). A similar "ecdysoneless" phenotype to *ecd-1* is produced by the $l(1)su(f)^{mad\text{-}ts}$ mutation (Klose et al. 1980), a temperature-sensitive cell lethal which also induces leg duplications in heat-pulsed flies (Jurgens and Gateff 1979). Heat-induced cell death during larval, prepupal and pupal development may therefore account for some or all of the morphological defects in *ecd-1* flies. Arking (1975) has suggested that the extent of cell death in some or all cell lethals is related to the level of mitotic activity. This is consistent with our finding that: i) the highest frequency of defects is produced by heat-pulsing *ecd-1* larvae in mid-larval life; ii) bristle and abdominal defects result from heat treatment during prepupal and early pupal development, coinciding approximately with the period of bristle-precursor cell and histoblast divisions (Poodry 1975).

Table 3. The transplantation of ovaries between OrR and *ecd-1* females. Donor ovaries, dissected within 2 h of emergence at 18° C, were implanted into 3-day old hosts and examined after 5–6 days at 29° C

Donor	Host	No. implants	No. donor ovaries developing beyond stage 8	No. donor ovaries with degenerate vitellogenic stages	Average[b] no. oocytes in each stage						
					8[a]	9	10	11	12	13	14
ecd-1	OrR	15	15	15	+	–	–	–	–	–	2.4
OrR	ecd-1	13	9	1	+	1.0	0.8	–	0.1	0.3	2.6
ecd-1	ecd-1	15	15	15	–	–	–	–	–	–	0.2
OrR	OrR	12	7	0	+	0.4	0.1	–	0.3	0.1	1.6

[a] Only the presence (+) or absence (–) of stage 8 oocytes was recorded
[b] Averages are calculated only from donor ovaries with oocytes developing beyond stage 8

The small size of the ring gland and larval salivary glands, tissues consisting mainly of polytene cells, suggests that as well as causing cell death, perhaps by acting on cells in mitosis, the *ecd-1* mutation also inhibits polytene cell growth cycles. The low rate of ecdysteroid secretion by *ecd-1* ring glands may be attributable to this rather than to specific effects on ecdysteroid synthesis or its regulation. Larval tissues, but not necessarily the ring gland (Redfern 1983), may be responsible for the increase in ecdysteroid titre during pupal development. These tissues will be near their maximum size at the time of pupariation and, if it is true that the low rate of ecdysteroid synthesis in non-pupariating *ecd-1* larvae is due solely to lack of tissue growth, the restrictive temperature will not prevent a subsequent increase in ecdysteroid titre, as we have found (and see Marsh and Wright 1982).

Sterile phenotypes are also common among temperature-sensitive cell lethals (Arking 1975). Two defects are apparant in adult *ecd-1* females at the restrictive temperature: defective chorionogenesis and the failure of new follicles to enter vitellogenic stages of oocyte maturation. Unlike Audit-Lamour and Busson (1981), we attribute these effects to defective follicle epithelial cells rather than to the reduced ecdysteroid levels present in these females. Follicle epithelial cells of *Locusta* produce ecdysteroids for storage in the egg (Lagueux et al. 1977; Hoffmann et al. 1980) and, although the evidence for ecdysteroid accumulation in the ovaries and eggs of *Drosophila* (Garen et al. 1977; Handler 1982) is at best equivocal, it is possible that the reduced titres in *ecd-1* females is a result of defective follicle epithelial cells.

Somatic-cell-limited effects are responsible for the production of flaccid, collapsed eggs (a similar phenotype to *ecd-1* eggs laid after a transfer to the restrictive temperature) in most female-sterile mutations studied by Weischaus et al. (1981) and this phenotype presumably results from defective follicle epithelial cells. We therefore presume that follicle epithelial-cell function is disrupted during chorionogenesis in *ecd-1* females at the restrictive temperature. These cells are mitotically active earlier in oogenesis (King 1970) and, by analogy with the effects of a heat pulse during larval and pupal development, may also be heat sensitive at this stage, preventing the initiation of vitellogenic development. Follicle epithelial-cell death in the $l(1)su(f)^{76a\text{-}ts}$ cell lethal results in an amorphous arrangement of nurse-like cells and no follicle epithelium (Wilson 1980). The ovaries of *ecd-1* females at the restrictive temperature do not have such an extreme morphology and since we have not observed cell death in the follicle epithelium we must assume that the two types of ovarian defects in *ecd-1* females result from non-lethal effects on follicle epithelial-cell function.

The ovarian defects clearly do not result from a lack of yolk-proteins. Yolk-protein synthesis has been postulated to be controlled by 20-hydroxyecdysone in *Drosophila* (Jowett and Postlethwait 1980; review: Bownes 1982). However, if haemolymph as well as whole fly ecdysteroid titres are reduced in *ecd-1* females (there is no firm evidence for this) the fact that yolk-protein synthesis continues at the restrictive temperature, even in females transferred to 29° C after the pupal temperature-sensitive period, suggests that ecdysteroids play no part in regulating or maintaining yolk-protein synthesis.

The *ecd-1* mutation thus produces a range of defects at the restrictive temperature. Similar morphological and developmental defects have been described in temperature-sensitive cell lethal mutants (Russell 1974: Dudick et al. 1974; Arking 1975; Simpson and Schneiderman 1975; Russell et al. 1977; Klose et al. 1980) and in the case of *ecd-1* these effects may result from the temperature-sensitivity of a more general cellular function than ecdysteroid synthesis, resulting in autonomous or 'mosaic' pleiotropy (Stern and Tokunaga 1968). Attempts to interpret functional deficiencies of *ecd-1* animals at the restrictive temperature in terms of hormone deficiency should therefore be viewed with caution.

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