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Analysis of Sex Determination in the Monogenic Blowfly *Chrysomya rufifacies* by Pole Cell Transplantation

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Summary. Sex determination in the monogenic blowfly *Chrysomya rufifacies* is controlled by a dominant or epistatic female sex realizer (F') having sex predetermining properties (F'/f = female-producing female; f/f = male-producing female or male, respectively). To determine (1) the cell type in which the maternal effect gene F' is expressed, and (2) the autonomous or nonautonomous sexual differentiation of the germ cells germ-line mosaics were constructed in *C. rufifacies* by pole-cell transplantations. The production of bisexual progeny by germ-line mosaics generated by transplanting pole cells between both types of female embryos shows that the F' gene product is synthesized by germ-line cells themselves, not by maternal (intra- or extraovarian) somatic cells. Pole cell transplantations between male and female embryos yielded completely fertile heterosexual germ-line mosaics thus demonstrating phenotypic sex reversal of donor germ cells in a host of the opposite sex. Consequently, the sexual differentiation of a germ cell in *C. rufifacies* is not determined by its own genotypic constitution but is induced by the surrounding somatic cells.

The male sex of F'/f individuals generated by fertilization with F'-bearing sperm from a heterosexual germ-line mosaic indicates that the F' gene is either not expressed during spermatogenesis and early embryogenesis or is expressed too late or in not sufficient amounts to direct differentiation into the female sex. This finding is consistent with the assumption that progamic expression of F' is found exclusively during oogenesis in F'/f females.

Introduction

The strictly monogenic reproduction in the blowfly *Chrysomya rufifacies* (Roy and Siddons 1939; Ullerich 1963) is caused by a special mechanism of sex determination: Female-producing (thelygenic; t) females are heterozygous for a dominant or epistatic female sex realizer F' having a sex-predetermining effect, while male-producing (arrhenogenic; a) females as well as males are homozygous for the recessive allele f (Ullerich 1973, 1975). This mode of sex determination offers an opportunity to gain further insight into the processes of sex determination and sexual differentiation in animals.

The maternal effect of the sex realizer, F', in *C. rufifacies* indicates that the female-determining gene product has to be synthesized during oogenesis of the t-females. The F' gene product could be produced in the cells of the germ line or in maternal somatic cells and transported into the growing oocytes. Recently performed ovary transplantation experiments have shown that the ovarian anlagen in *C. rufifacies* at least from the young third instar stage, are autonomous for the product of the F' gene (Ullerich 1980, 1981). These results suggest an intraovarian synthesis of that gene product. Within the ovary, the F' gene could be expressed either in the germ line itself or in the somatic, mesodermally derived components of the gonads (follicle epithelium, ovarian sheath). This can be decided by the construction of germ-line mosaics in *C. rufifacies* by transplanting primordial germ cells between a-type and t-type embryos following the method first developed in *Drosophila* (Illmensee 1973). In *Drosophila* and several other insects the primordial germ cells, designated pole cells, are formed as the first cells of the syncytial blastoderm embryo at its posterior pole; during gastrulation they invaginate and migrate to the gonads (for review, see Anderson 1972).

Pole-cell transplantation experiments have proved to be a powerful technique for determining the developmental fate of the transplanted cells in *Drosophila* (Illmensee 1978). By this procedure germ-line autonomy of some maternal-effect mutations of *Drosophila* has been shown recently (see Discussion). From the occurrence of exclusively homosexual germ-line mosaics after transplanting at random pole cells between male or female donors and male or female recipients it has been concluded that the phenotypic sex of a germ cell in *Drosophila* is determined entirely by its own chromosomal constitution, not by that of the gonadal soma (van Deusen 1976). The absence of heterosexual germ-line mosaics suggests that donor pole cells cannot reverse their phenotypic sex and form functional gametes in a host of the opposite sex (Illmensee 1973; van Deusen 1976; Marsh and Wieschaus 1978). These findings, however, do not exclude a somatic control of germ-cell differentiation in insects. They are consistent with the hypothesis that the gonadal soma initiates sexual differentiation of the germ cells, but that their ability to respond to this impetus is limited by the genotype, i.e., the sex chromosome constitution of the germ cells themselves.

In contrast to *Drosophila* and many other insects, *C. rufifacies* possesses no heteromorphic sex chromosomes (Ullerich 1963). Probably t-females on the one hand and a-females and males on the other differ genetically only by the presence or absence, respectively, of the sex realizer F'. Therefore, in heterosexual host/donor combinations of

C. rufifacies phenotypic sex reversal of donor pole cells might occur. Thus, *C. rufifacies* seems to be a suitable experimental system to test whether or not the sexual development of germ cells depends primarily on signals from the surrounding somatic cells.

To determine (1) the intraovarian cell type in which the maternal effect gene *F'* is expressed, and (2) the autonomous or nonautonomous sexual differentiation of the germ cells, two series of pole-cell transplantations were performed in *C. rufifacies*. First results of these experiments have been published recently (Ullerich 1982, 1983). In this paper, following a brief presentation of scanning electron microscopic findings on pole-cell formation in *C. rufifacies*, the results of the complete series of the transplantation experiments are described.

Materials and Methods

Fly Strains. The experiments were performed with individuals from a wild-type stock (+/+) of *Chrysomya rufifacies* (Diptera: Calliphoridae, Calliphorinae) and from a mutant stock carrying the recessive sex-linked marker *white* (*w/w*; white eyes, white Malpighian tubules (Ullerich 1973, 1980)). Both strains of *C. rufifacies* have been kept in the laboratory for many years (Ullerich 1973).

Breeding Conditions. Flies were commonly reared at 25° C. Breeding and crossing were the same as described previously (Ullerich 1963) with only slight modifications (Ullerich 1973).

Scanning Electron Microscopy of Pole Cells. At 10–30 min intervals embryos of the wild-type stock were collected and prefixed in Karl's solution (10 parts distilled water, 5 parts 96% ethanol, 2 parts 37% formaldehyde, 1 part glacial acetic acid; see Jung 1966) at 60° C for 2 h. Chorion and vitelline membrane were removed and the embryos left in fresh Karl's fixative overnight at room temperature. Then the embryos were dehydrated through a graded series of ethanols, dried by the Frigen procedure, coated with gold, and observed in a Cambridge Stereoscan S4-10 microscope.

Pole Cell Transplantation. Essentially the pole-cell transplantation procedure described by Illmensee (1973) for *Drosophila* was followed with only slight modifications. Donor and recipient eggs were harvested 60–90 min after deposition, dechorionated in 2.5% Na-hypochlorite, washed extensively with distilled water, and stored until the blastoderm stage on filter paper. Immediately prior to transplantation the embryos were allowed to dry to reduce turgor (20 min at 28° C). After desiccation the donor and recipient embryos were placed for transplantation into the operation chamber containing an oil medium. This was prepared by mixing sterilized Ringer's solution and paraffin oil (2:1) with a magnetic stirrer for 24 h; the suspension was then centrifuged (500 *g*), and the supernatant oil phase was used as operation medium. The transplantations were carried out with glass micropipettes using a Leitz-Micromanipulator set. The micropipette holding the egg at its anterior pole as well as the injection pipette was attached to a paraffin oil-filled syringe system; the injection pipette was filled with Ringer's solution. As many pole cells as possible (usually 5–25) were removed from a donor and transferred to a single host among its own ones. Pole cells were transplanted between late blastoderm up to early gastrula stages. Some minutes after this operation the recipient eggs were transferred with a fine brush to dry filter paper to remove most of the adhering oil; this procedure promotes the survival of the embryos. Thereafter the recipients were kept in a moist chamber (Ringer's solution) overnight at 28° C, the hatched larvae were placed on the standard food and reared at 25° C.

Donors and Recipients. All transplantation experiments were performed by using *w/w*-marked donors and +/+ recipients. Male embryos cannot be distinguished from female ones; therefore, germ-line mosaics were produced by transplanting at random pole cells between the sexes. Since males, a-type and t-type females in the strains of *C. rufifacies* occur in the ratio of approximately 1/2:1/4:1/4 (Ullerich 1963), theoretically the following homo- and heterosexual host/donor combinations can be expected: ♂/♂, ♂/a-♀, ♂/t-♀, a-♀/♂, t-♀/♂, a-♀/a-♀, t-♀/t-♀, a-♀/t-♀, and t-♀/a-♀ in a ratio of 1/4:1/8:1/8:1/8:1/8:1/16:1/16:1/16:1/16.

In the first transplantation series (A) both donor and recipient embryos were taken from mass cultures. To identify reliably heterosexual germ-line mosaics, a second transplantation series (B) was performed. For these experiments recipient male and female embryos were also taken at random from mass cultures. But as a source of donor pole cells several embryos from the unisexual progeny of single females were used; the sex of the donors was identified later after eclosing of the residual individuals from those progenies.

Test Mating. The adult recipients (+/+) were test-mated to *w/w* flies. Succesful development of donor pole cells (*w/w*) is indicated by the appearance in the progeny of white-eyed animals (*w/w*) besides the heterozygous wild-type flies (+/*w*) arising from the host's own germ cells. Male hosts were usually test-mated to several (a-type and t-type) females thus giving on the average a larger number of (male and female) offspring than female hosts. To test the (thelygenic) type of host or donor females, some of the daughters of several germ-line mosaic females were outcrossed; the production of all-female progeny by at least one daughter of a mosaic female indicates the thelygenic character of the host or donor, respectively (see Tables 2 and 3).

Results

Pole Cell Formation

Histological investigations and observations on living embryos have shown that pole cell formation in *C. rufifacies* resembles that in *Drosophila* and other diptera (Scheel 1981). This is confirmed by scanning electron microscopic (SEM) studies on *C. rufifacies* eggs (Fig. 1 a–i). The egg of this species is about 1.2 mm long and 0.3 mm wide. Its anterior and posterior poles can be distinguished by the somewhat larger diameter of the posterior tip. The syncytial blastoderm stage begins about 90–100 min, the cellular blastoderm stage 140–150 min, and the gastrulation 180–200 min after egg deposition at 25° C. During early syncytial blastoderm 15 to 20 pole cells emerge at the posterior pole of the embryo (Fig. 1 b, c). The young pole cells possess numerous cytoplasmic projections which later re-

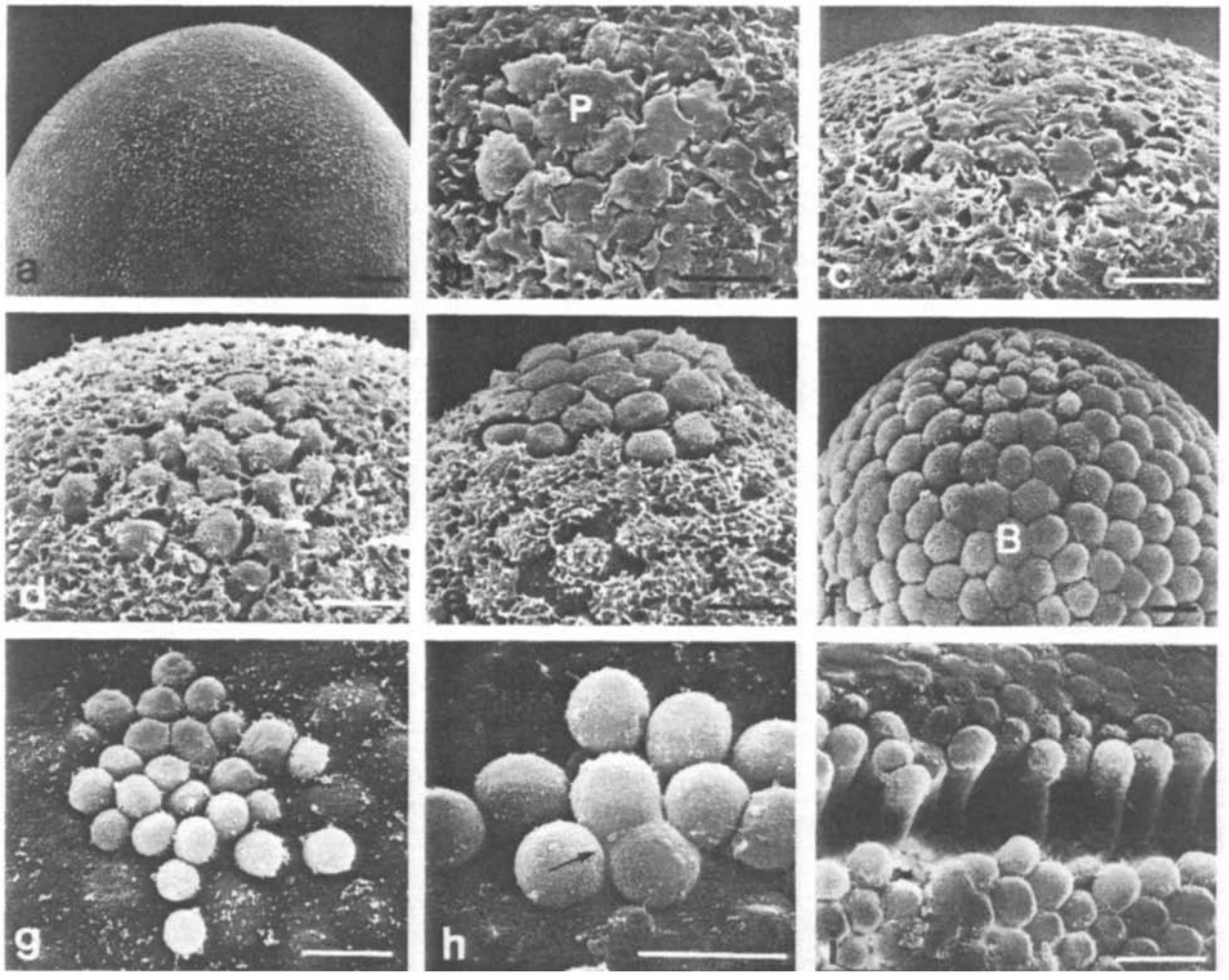

Fig. 1 a–i. SEM pictures illustrating pole cell formation. **a** Posterior pole of a preblastoderm embryo (0.5 h). **b–d** Formation of the first pole cells during syncytial blastoderm stages (1.5–2 h). **e** Beginning of formation of blastoderm cell membranes (2 h). **f** Early cellular blastoderm stage (2.5 h). **g** Pole cell complement of a blastoderm embryo (3 h). **h** Division of a pole cell (arrow). **i** Fracture through the blastoderm layer (late blastoderm stage) demonstrating the columnar blastoderm cells. *P:* Pole cells, *B:* blastoderm cells. Bars indicate 20 μm

gress (Fig. 1 b–e). In the subsequent state the pole cells are, in contrast to the columnar blastoderm cells (Fig. 1 i), almost spherical and have a diameter of 10–11 μm (Fig. 1 f–h). During the syncytial blastoderm stage some of the initially formed pole cells divide asynchronously (Fig. 1 h). Thus, when the cellular blastoderm is completed (Fig. 1 f, g), a cluster of 24–30 pole cells is present which becomes internal within the first 30 min of gastrulation.

The number of pole cells observed in *C. rufifacies* is lower than the mean number found in *Drosophila* (Turner and Mahowald 1976) but agrees well with that known from the closely related calliphorid species *Lucilia sericata* (Davis 1967). The SEM findings on pole cell formation in *C. rufifacies* correspond largely with those recently described in more detail for *Drosophila* (Turner and Mahowald 1976).

Germ Line Autonomous Expression of the F' Gene

The developmental rates of the +/+ recipient embryos injected with pole cells from *w/w*-marked donors of undetermined sex (experimental series A) and from sex-controlled *w/w* donors (experimental series B) are summarized in Table 1. As expected, only a small minority of the manipulated hosts reached the adult state. For technical reasons, most of them died as embryos or larvae. The higher survival rate in series B is certainly due to increasing experience and technical improvements in the course of the experiments. Between a third and half of the fertile hosts test-mated to *w/w* flies turned out to be germ-line mosaics (Ta-

Table 1. Developmental rates of +/+ host embryos injected with *w/w* donor pole cells in experimental series A and B

Exp. series	Host embryos injected	Hatched larvae	Pupae	Imagos					
				Hatched		Fertile		Mosaics	
				♀♀	♂♂	♀♀	♂♂	♀♀	♂♂
A	1619	657 (40.6%)	157 (9.6%)	63 (6.7%)	45	36 (4.0%)	29	14 (1.5%)	11
B	929	523 (56.3%)	145 (15.6%)	49 (9.5%)	39	35 (6.9%)	29	20 (3.4%)	12

Table 2. Progeny of mosaics constructed by transplantation of pole cells: Experimental series A

♀♀ mosaics		Phenotype and number of progeny			
Inferred type (host/donor)	Number	Wild-type (+/w)		White (w/w)	
		♀♀	♂♂	♀♀	♂♂
t-♀/t-♀	1	324	–	137 (16t/21a)[a]	–
a-♀/a-♀ or ♂	6	–	78	–	11
		–	391	–	5
		–	149	–	104
		–	147	–	56
		–	113	–	111
		–	189	–	12
t-♀/a-♀ or ♂	6	179 (29t/23a)[a]	–	–	55
		205 (22t/26a)[a]	–	–	199
		131	–	–	98
		92	–	–	78
		60	–	–	5
		59	–	–	4
a-♀/t-♀	1	–	58	3 (2t)[a]	–
♂♂ mosaics (total)	11	3079	3004	555	362

[a] In parantheses: Number of t-type and a-type females identified among that progeny

ble 1), as proven by the appearance of a number of white-eyed (*w*/*w*) individuals beside their heterozygous (+/*w*) wild-type progeny (Tables 2, 3). The different high portion of white-eyed animals in the progenies of the various germ-line mosaics certainly reflects the higher or lower number of donor pole cells transplanted and successfully incorporated by the host.

The progeny of germ-line mosaics and the inferred types of host/donor combinations obtained in experimental series A are presented in Table 2. Beside 11 male mosaics 14 female ones were found. One of these females produced exclusively daughters thus disclosing the thelygenic character of the host (*F'*+/*f*+); the presence of t-type and a-type animals among the white-eyed daughters as revealed by outcrossing some of them shows that the donor also was a t-female (*F'w*/*fw*). Six mosaic females gave rise to unisexual male broods; consequently, these recipients were a-females (*f*+/*f*+) injected with pole cells either from an a-female or – if phenotypic sex reversal of the implanted pole cells did occur – from a male donor, respectively (*fw*/*fw*). Six further mosaic females yielded wild-type daughters and white-eyed sons indicating that the thelygenic hosts (*F'*+/*f*+) had successfully incorporated pole cells from a-female (or male) *fw*/*fw* donors. The offspring of the last mosaic female of series A consisted of wild-type males and white-eyed females. This recipient female thus proved to have been arrhenogenic (*f*+/+) with implanted pole cells from a t-female donor (*F'w*/*fw*); the t-type of the donor is verified by the occurrence of t-females among the white-eyed progeny of that mosaic female (Table 2).

Further a-♀/t-♀ and t-♀/a-♀ host/donor combinations

Table 3. Progeny of mosaics constructed by transplantation of pole cells: Experimental series B

Mosaics			Phenotype and number of progeny			
Sex	Combination host/donor	Ser. no.	Wild-type (+/w)		White (w/w)	
			♀♀	♂♂	♀♀	♂♂
♀♀	t-♀/t-♀	1	117	–	7 (3t/2a)[a]	–
		2	90	–	83 (9t/8a)[a]	–
		3	67	–	15 (3t/6a)[a]	–
		4	163	–	23 (5t/5a)[a]	–
		5	95	–	57	–
		6	74	–	70	–
	a-♀/a-♀	7	–	130	–	7
	a-♀/t-♀	8	–	175	37 (3t/4a)[a]	–
		9	–	203	39 (12t/8a)[a]	–
		10	–	8	1	–
	t-♀/a-♀	11	88 (19t/19a)[a]	–	–	16
	a-♀/♂	12	–	329	–	2
		13	–	150	–	66
		14	–	198	–	37
		15	–	205	–	3
		16	–	95	–	60
	t-♀/♂	17	76	–	–	22
		18	258	–	–	12
		19	283	–	–	59
		20	11	–	–	6
♂♂	♂/t-♀	21	505	341	10	13
		22	221	184	313	194
		23	244	190	15	–
	♂/a-♀	Not obtained				
	♂/♂	24–32	3026	2822	1110	228

[a] In parantheses: Number of t-type and a-type females identified among that progeny

with corresponding bisexual progeny have been identified among those germ-line mosaics which were constructed in experimental series B (mosaic nos. 8 to 11, Table 3). The appearance of males (females) stemming from the host's own germ cells and females (males) developing from the donor germ cells in the same brood of a-♀/t-♀ and t-♀/a-♀ mosaics, respectively, clearly demonstrates that the sex realizer *F'* is expressed autonomously in cells of the germ line, independently from the genotype of intra- (and extra-)ovarian somatic tissues. This situation is diagrammatically represented in Fig. 2.

Functional Heterosexual Germ Line Mosaics

Beside the heterotypic female combinations mentioned above and some homotypic t-♀/t-♀, a-♀/a-♀ and ♂/♂ mosaics (mosaic nos. 1 to 6, 7, 24 to 32; Table 3), different types of heterosexual germ-line mosaics were detected in experimental series B. Heterosexual host/donor combina-

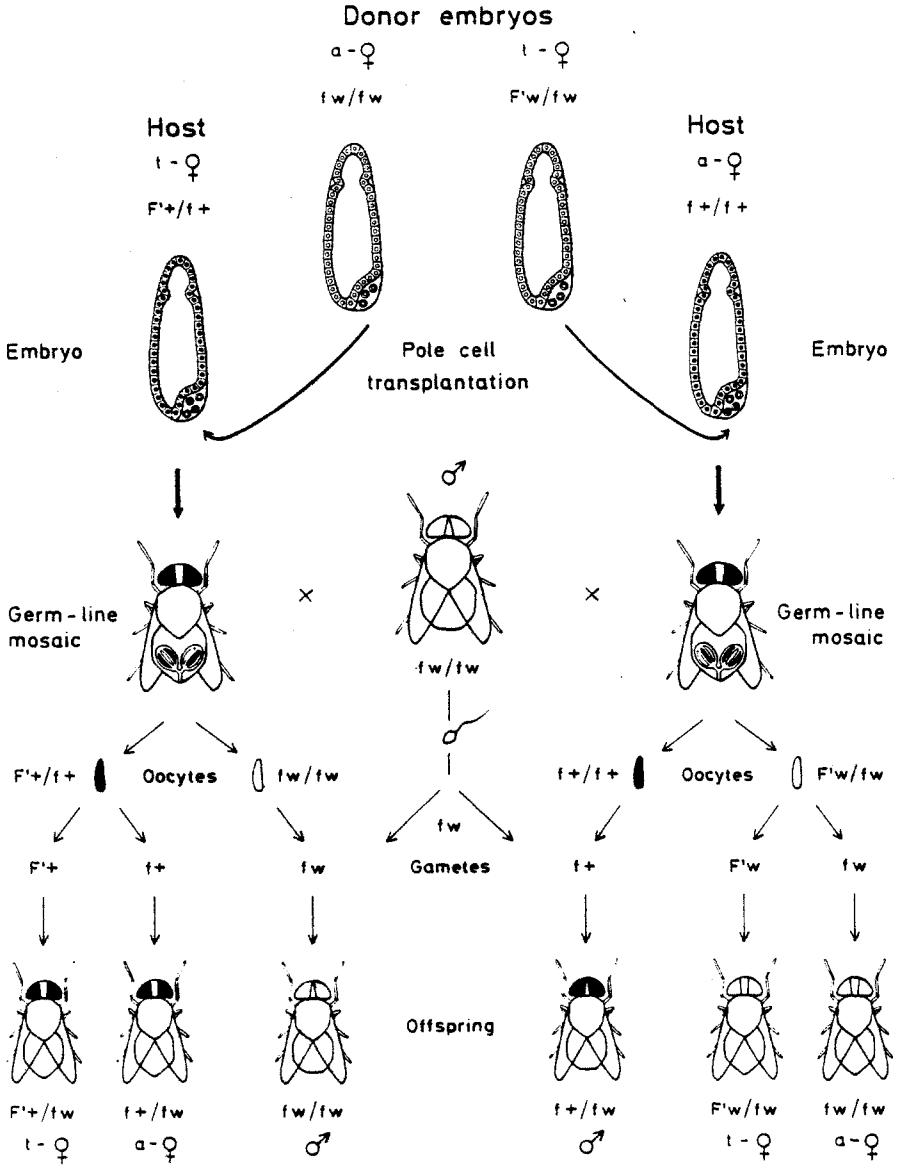

Fig. 2. Schematic illustration of the results of reciprocal pole-cell transplantations between thelygenic (*F'/f*) and arrhenogenic (*f/f*) female embryos in *Chrysomya rufifacies*. Donors were marked by *w* (*F'w/fw* and *fw/fw*, respectively), hosts were normal (*f+/f+* and *F'+/f+*, respectively). a-♀: Arrhenogenic female; t-♀: thelygenic female; *F'*: predetermining dominant female sex realizer; *f*: its recessive allele; *w*: *white* allele; +: w^+ allele

tions can be identified in *C. rufifacies* by using sex-controlled donors (see Materials and Methods); the ability of such mosaics to produce offspring also from donor germ cells is evidenced again by the appearance of white-eyed individuals among their progeny. Five arrhenogenic (*f+/f+*) and four thelygenic (*F'+/f+*) host females which had received pole cells from male donors (*fw/fw*) yielded white-eyed sons (*fw/fw*) beside the heterozygous wild-type sons or daughters, respectively, originating from the host's own germ cells (mosaic nos. 12–16, 17–20, Table 3). These findings show that primordial germ cells successfully transferred from male donor embryos to female recipients reverse their prospective sexual differentiation and form functional eggs in a-type as well as in t-type host females. The production of wild-type females and white-eyed males by the t-♀/♂ type of heterosexual germ-line mosaics (nos 17–20, Table 3) again confirms the germ-line autonomous expression of the *F'* gene.

Moreover, three heterosexual germ-line mosaics constructed by transplanting pole cells from female donors into male hosts were found (mosaic nos. 21–23, Table 3). All these host males – in consequence of test-crossing with several (t-type and a-type) females separated for egg deposition – gave rise to all-female and all-male progenies (combined for each host male in Table 3). Thus two of them produced wild-type and white-eyed offspring of both sexes; the third host male – presumably because it had incorporated only few donor pole cells – yielded white-eyed offspring among its female descendants only. The occurrence of white-eyed animals among the progeny of these heterosexual ♂/♀ germ-line mosaics clearly indicates that primordial germ cells deriving from female donors reverse their phenotypic sex in a male host and develop functional sperms.

Tests for Sex Reversal in Transplanted F'/f Germ Cells

Because of the genotypic identity of males and a-females (*f/f*) it has to be expected that sex reversal of donor pole cells in heterosexual mosaic males does occur at least in ♂/a-♀ combinations. To test whether pole cells from t-type donor females can also reverse their phenotypic sex in a male host, the white-eyed offspring of heterosexual male germ-line mosaics were outcrossed as shown in Figs. 3 and 4. If a male host (*f/f*) forms functional sperms from pole

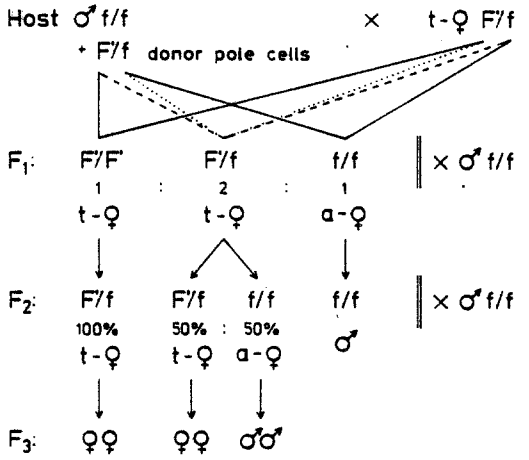

Fig. 3. Test scheme for identifying heterosexual germ-line mosaics of the host/donor combination ♂/t-♀ by outcrossing of F_1 individuals arising from donor germ cells. Signs and symbols as in Fig. 2

Table 4. Outcrossing of w/w F_1 females of the heterosexual germ line mosaics ♂/t-♀ nos. 21, 22, and 23 (see Table 3)

Mosaic no.	Number of F_1 ♀♀ tested	Sex and numbers of progenies					
		F_2 from		F_3 from			
		Single matings		Single matings		Mass matings	
		all ♀	all ♂	all ♀	all ♂	all ♀	♀♀+♂♂
21	8	6 (5)[a]	2			2	3
22	63	44 (39)[a]	19			12	27
23	3	3 (3)[a]	–	19	–		
				19	–		
				8	17		

[a] In parantheses: Number of all-female F_2 progenies from which F_3 progenies were reared

cells stemming from a thelygenic donor (*F'/f*), then its white-eyed female F_1 offspring resulting from test-crossing with a t-female theoretically should consist of homozygous thelygenic (*F'/F'*), normal heterozygous thelygenic (*F'/f*) and arrhenogenic (*f/f*) individuals in a ratio of 1:2:1 (Fig. 3). In contrast to their *F'/f* sisters producing t- and a-type daughters in nearly equal numbers, fertile *F'/F'* females should give rise to exclusively heterozygous, thelygenic F_2 females (*F'/f*), as can be tested by identifying the sex of their F_3 progenies (Fig. 3). The results obtained by outcrossing a portion of the *w/w* F_1 daughters of the ♂/t-♀ mosaics (nos. 21 to 23, see Table 3) are summarized in Table 4. The progeny of each F_1 female were reared separately. From a total of 74 *w/w* F_1 females examined 53 turned out to be thelygenic and 21 arrhenogenic (Table 4). Most of the F_2 progenies obtained were further tested. They were reproduced either by mating a number of isolated females from progeny (mosaic no. 23, Table 4) giving exclusively all-female or both all-female and all-male F_3 progenies, or by mating all females from progeny in mass culture (mosaic nos. 21 and 22, Table 4) giving purely female or bisexual F_3 progenies, depending on the presence of only t-females or both t- and a-females in the F_2 progeny in question.

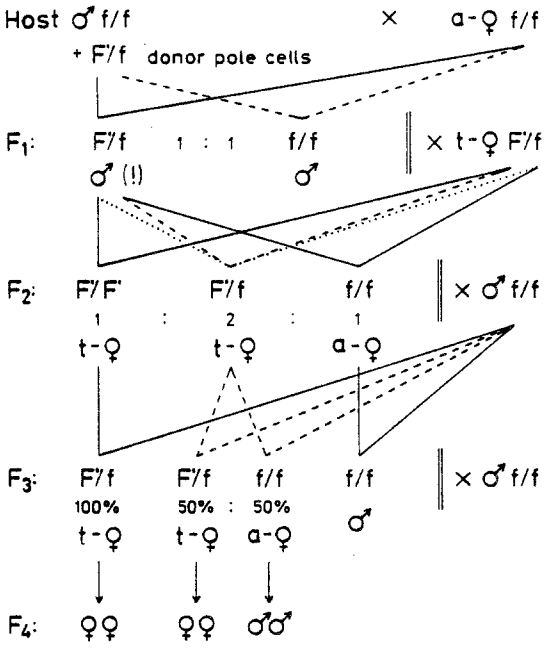

Fig. 4. Test scheme for identifying *F'/f* males among the F_1 progeny of a ♂/t-♀ germ-line mosaic crossed with an a-female. Signs and symbols as in Fig. 2

Progenies consisting of exclusively t-females were found among the F_2 progenies of each of the three ♂/♀ mosaics tested, as indicated by the occurrence of corresponding all-female F_3 progenies (Table 4). The occurrence of exclusively all-female progenies derived from 16 out of 47 examined female F_2 progenies indicates that approximately a third of the thelygenic *w/w* F_1 individuals must have been homozygous *F'/F'* (Table 4). These numbers of thelygenic *F'/F'* and *F'/f* females (16:31) give an excellent approximation of the expected 1:2 ratio (Fig. 3). In addition, the appearance of *F'/F'* females agrees well with the high proportion of t-females compared to a-females among the *w/w* F_1 individuals (53:21; χ^2 test for a 3:1 ratio: $P=0.5$). The existence of *F'/F'* females in the F_1 progeny of all three ♂/t-♀ mosaics shows that each of these male hosts had incorporated pole cells from a thelygenic donor female and formed functional *F'*-bearing sperms beside the *f*-bearing ones. The ♂/a-♀ type of host/donor combination could not be detected among the germ-line mosaics obtained in experimental series B.

The ♂/t-♀ type of germ-line mosaic, if mated to an *arrhenogenic* female (*f/f*), should produce *F'/f* and *f/f* offspring from *F'/f* donor germ cells in nearly equal number (Fig. 4). The presence of only male white-eyed offspring in the progeny arising from crosses of that kind suggested that the resulting *F'/f* zygotes develop into males not, as normally, into t-females. To identify those *F'/f* individuals, the *w/w* sons of one of the ♂/t-♀ mosaics (no. 21, see Table 3) were outcrossed as represented diagrammatically in Fig. 4. If those *F'/f* individuals were really fertile males, after mating with t-females they should have produced homozygous *F'/F'* females in addition to normal *F'/f* and *f/f* females. The appearance of *F'/F'* females (in F_2) can be inferred again, as described above, by the occurrence of all-female (F_4) progenies after outcrossing of female F_2 and F_3 progenies (Fig. 4). The results of these crosses are summarized in Table 5. Out of 13 *w/w* sons of that ♂/t-♀

Table 5. Outcrossing of w/w F_1 males of the heterosexual germ line mosaic ♂/t-♀ no. 21 (see Table 3)

F_1 ♂♂ with female F_2 progeny	Sex and numbers of progenies					
	F_3 from		F_4 from			
	Single matings		Single matings		Mass matings	
	all ♀	all ♂	all ♀	all ♂	all ♀	♀♀+♂♂
♂1	7 (7)[a]	3			–	7
♂2	5 (4)[a]	5			–	4
♂3	7 (7)[a]	2			3	4
♂4	14 (10)[a]	–	25	–		
			13	7		
			9	14		
					2	5
♂5	12 (10)[a]	3	13	–		
			15	–		
					3	5

[a] In parantheses: Number of all-female F_3 progenies from which F_4 progenies were reared

mosaic (no. 21, Table 3) five produced – in consequence of mating with t-females – female F_2 progeny (not shown in Table 5). A portion of each F_2 progeny was reproduced by single matings giving a number of all-female and all-male F_3 progenies (Table 5). Most of the female F_3 progenies were outcrossed, either by mass matings (see F_1 males nos. 1, 2, and 3, Table 5) or by both single and mass matings (see F_1 males nos. 4 and 5, Table 5). The appearance of some all-female F_4 progenies derived from the F_1 males nos. 3, 4, and 5 discloses the presence of F'/F' females in the corresponding F_2 generations and thus demonstrates that those F_1 males must have possessed the heterozygous constitution F'/f, compared to their brothers nos. 1 and 2 which might have been common f/f males (Table 5). These findings show that egg cells of arrhenogenic females fertilized by F'-bearing sperms do not develop into t-females but into phenotypically normal and fertile males.

Discussion

Maternal effects are due to the storage of gene products in the oocyte which are needed during embryogenesis (for review, see Davidson 1976). Maternal-effect genes are known from several animals, but in most cases it is unknown which of the maternal cells synthesize the predetermining gene product. This may be the oocyte itself, as has been reported for the o^+ gene of the Mexican axolotl, *Ambystoma mexicanum* (Briggs and Justus 1968) and the or^+ gene of the polychaete *Platynereis dumerilii* (Fischer 1977), or maternal somatic cells as known from the a^+ gene in the moth *Ephestia kühniella* (Kühn et al. 1935).

In the case of the female sex realizer F' in *Chrysomya rufifacies*, the experiments described in this report demonstrate that its gene product is synthesized by the germ line. This is evidenced by the production of both sexes by germline mosaics generated by reciprocal pole-cell transplantations between a-type and t-type female embryos; such mosaics show that the sexual development of their offspring deriving from donor germ cells is independent from the sex of the host and follows the genotypic constitution of the donor. If the F' gene product had been synthesized in (intra- or extraovarian) somatic cells and transferred into the growing oocytes, the germ cells from arrhenogenic donors (f/f), developed in thelygenic hosts (F'/f), should have resulted in arrhenogenic females and not in males, as was the case. Among insects germ-line autonomy of maternal effect mutations has already been shown by pole-cell transplantations in *Drosophila* for *deep orange* (Marsh et al. 1977), *maroon-like* (Marsh and Wieschaus 1977), *fs(1)K10* (Wieschaus et al. 1978), *mat(3)1* (Regenass and Bernhard 1978), and *agametic* (Engstrom et al. 1982). The F' gene of *C. rufifacies* is the first sex realizer with maternal effect analyzed by pole-cell transplantations and behaves as germ-line-autonomous.

In insects with meroistic ovaries as in *Drosophila* and *Chrysomya* pole-cell transplantation experiments cannot elucidate the type of germ-line cells by which the predetermining gene product is synthesized. The F' gene product in *C. rufifacies* might be produced either in the oocyte or in the nurse cells and transported into the oocyte (for a detailed discussion of these alternatives, see Ullerich 1980). The recent demonstration of a transcriptionally active lampbrush phase at the end of pachytene in the oocytes of some *Drosophila* species and *Calliphora erythrocephala* (Dävring and Sunner 1982), the latter being closely related to *Chrysomya*, supports the hypothesis that the F' gene product in *C. rufifacies* might be synthesized in the oocyte itself.

The present study has further shown that in *C. rufifacies* a genotypically male germ cell does differentiate a functional oocyte in a female host, and conversely a genotypically female germ cell does develop a functional sperm in a male host. These observations disclose a nonautonomous sexual differentiation of the transplanted pole cells. Therefore, the primordial germ cells at the blastoderm and early gastrula stage in *C. rufifacies* are still not determined with respect to their future sex. This implies that the factors that regulate the sexual differentiation of the germ cells reside in the surrounding somatic cells.

In *Drosophila*, by contrast, germ-line sex reversal does not occur; the phenotypic sex of the germ line seems to be determined entirely by its own genotype (van Deusen 1976). A similar situation is assumed to exist in mammals where sex reversal has been shown to be limited largely to the soma. Partial gonadal sex reversal can be produced in marsupials; in eutherian mammals, in some cases sex reversal of germ cells could be achieved, but in no case has complete functional sex-reversed germ cells yet been obtained (see Mittwoch 1973; McCarrey and Abbott 1979; Chan and O 1981). Complete functional sex reversal can occur in fish, amphibia and birds. In the latter, varying degrees of germ-line sex reversal have been produced experimentally indicating a somatic influence upon the sexualization of the germ cells; however, in most cases functional gametogenesis has not been accomplished (see McCarrey and Abbott 1979). The relative ease of achieving sex reversal in amphibia and fish demonstrates that in these lower vertebrates, most of which having undifferentiated sex chromosomes (see Mittwoch 1973; Schmid 1983), the developmental fate of the germ cells is less rigidly controlled by their own genotype while the gonadal soma appears to be an important determining factor for the sexual differentiation of the germ cells (Chan and O 1981). In gonochoristic invertebrates, a similar situation consisting in the induction

of the male sex in the germ cells by means of somatic tissue does exist in some Crustacea, probably in all Malacostraca (see Bacci 1965). The general picture that emerges from the data concerning germ-cell differentiation in gonochorists is that the somatic components of the gonad initially induce sexual differentiation of the germ cells, but the extent of response to this impetus is dependent upon the genotype, i.e., the sex chromosome constitution of the germ-line cells themselves (McCarrey and Abbott 1979).

The results obtained in *C. rufifacies* support this hypothesis. Although *C. rufifacies* represents, as *Drosophila* too, a highly evolved insect species, its neo-X'X-XX (*F'f-ff*) mechanism of sex determination is still in a primitive state, the sex chromosomes being very little differentiated from autosomes, and hence not distinguishable morphologically (Ullerich 1975, 1976). Such minor genotypic differences between the sexes seem to enable the donor germ cells in heterosexual germ-line mosaics to respond to the somatic induction of sexual differentiation with complete functional sex reversal. Whereas in *Drosophila* sex determination in germ line and soma seems to be controlled by different sets of genes (Marsh and Wieschaus 1978), the soma-dependent sexual development of the germ cells in *C. rufifacies* suggests that in this species germ line and soma do not have separate genetic mechanisms for sex determination.

Among insects, somatic influence on sex of germ line apparently exists also in the monogenic sciarid fly *Sciara coprophila*. The X'X-XX mechanism of sex determination in this species resembles that in *C. rufifacies*, but is complicated by the presence of germ-line limited chromosomes and by directed segregation and elimination of chromosomes resulting in different sex chromosome constitutions in the soma of the sexes (XO = male, X'X = thelygenic female, XX = arrhenogenic female; Metz 1938). Cytogenetic studies support the view that the X' influence on sex of progeny is on sex chromosome elimination from the embryonic soma, and that the sex of the individual fly and, thus, of its germ line is determined by the chromosome complement which remains in the soma after elimination (Crouse 1960, 1965).

The appearance of functional *F'/f* males and *F'/F'* females in the progeny of heterosexual ♂/t-♀ germ-line mosaics of *C. rufifacies* is in accordance with the occurrence of exceptional X'O males in *S. coprophila* which produce fertile X'X' daughters when mated to normal X'X females (Metz and Schmuck 1929). It proves that the *F'*-bearing chromosome (X') can be substituted for its *f*-bearing partner (X) in both males and females without any conspicuous effect on vitality and fertility of the flies. This indicates a basic similarity in constitution between the homologues which may differ solely by the presence or absence, respectively, of the sex realizer *F'*. Thus the state of differentiation of the sex chromosomes in *C. rufifacies* is also comparable to that in most lower vertebrates (Becak 1983). The male sex of the above-mentioned *F'/f* individuals in *C. rufifacies* shows that the *F'* gene introduced by a sperm is either not expressed during spermatogenesis and early embryogenesis or is expressed too late or not in sufficient amounts to direct differentiation into the female sex. This finding underlines the predetermining effect of the *F'* gene and is consistent with the assumption that progamic expression of *F'* is found exclusively during oogenesis in *F'/f* females, as is already indicated by the formation of a-type females phenotypically identical to t-type females but genotypically males. Consequently, in *C. rufifacies* only one major (regulatory) sex gene (or a sequence of a few closely linked genes?) seems to be present, which decides the sexual differentiation of the individual at the earliest possible stage of development; the presence of the *F'* gene product in the oocyte results in female sex and its absence in male sex. This specific and relatively "simple" mode of sex determination in *C. rufifacies* appears favorable for use of the techniques of molecular biology to gain further insight into the processes of sex determination and sex differentiation.

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