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Gynandromorphs of *Drosophila* suggest one common primordium for the somatic cells of the female and male gonads in the region of abdominal segments 4 and 5

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Summary

In mosaic gonads of gynandromorphs of *Drosophila*, the amount of female and the amount of male somatic tissues add up to roughly one unit. This suggests that the somatic component of the gonads in males and females derives from a single common primordium, i.e. testes and ovaries appear to be homologous. Fate-mapping places this primordium ventrally of the sternites into the mesodermal region of the fourth and fifth abdominal segment. This location is corroborated by the observation that defects in and around abdominal segment 4 and absence of the gonads are strongly correlated in animals damaged by the mutation osk^{301}. Gonads were mosaic with a frequency of 10.5% which indicates that the gonadal primordium originates from about 10 progenitor cells, and together with other evidence, suggests that these progenitor cells are located within a single segment (or parasegment).

Key words: fate map, genetic mosaics, gonadal development, sex determination, sexual dimorphism, *Drosophila*.

Introduction

The gonads of *Drosophila melanogaster* are composite organs consisting of mesodermal somatic tissue and of the germ line. These two components originate from different progenitor cells located in different regions of the embryo. The so-called pole cells are the precursors of the germ cells. They form at the posterior pole of the embryo from where they are moved dorsally and anteriorly. They subsequently migrate inside the embryo and enter the mesoderm where they eventually become engulfed in the mesodermal gonadal anlage. Prospective germ cells and mesodermal somatic cells develop in intimate contact and mutually influence each other (Steinmann-Zwicky et al. 1989; Nöthiger et al., 1989). The formation of a mature gonad with functional germ cells thus requires coordinated gene expression in the germ line and in the somatic cells.

Whereas origin and development of the germ cells are well studied (Sonnenblick, 1941, 1950; Illmensee and Mahowald, 1974; Underwood et al., 1980; Technau and Campos-Ortega, 1986; Hay et al., 1988; Lasko and Ashburner, 1990), we are largely ignorant about the precursors and the development of the somatic component of the gonads. In particular, we do not know whether testes and ovaries are formed from one single pool of precursor cells or from different precursor pools. We also lack information about the size of the gonadal primordium and from which abdominal segment or segments the anlage derives (see Campos-Ortega and Hartenstein, 1985).

We decided to address these questions by studying gynandromorphs. We quantified the relative amounts of testicular and ovarian tissue in mosaic gonads of gynandromorphs, and we mapped the gonadal primordium. Our results suggest that the somatic parts of the gonad of *Drosophila* originate from a single primordium of about 8-10 cells, and that one such primordium is located ventrally in the mesodermal region of the fourth and fifth abdominal segment on each side of the embryo.

Materials and methods

Crosses and genotypes

Regular gynandromorphs (with germ line cells) were generated by crossing *y v f mal* homozygous females with *Fs(3)Horka/TM3* males. The mutations *y*, *v*, *f* and *mal* are X-linked recessive marker mutations that allow identification of the genotype of the cuticle (*y* and *f*) and of internal organs (*mal*; Janning, 1978). *Fs(3)Horka* is a dominant female-sterile

mutation (Erdélyi and Szabad, 1989). X-chromosomes that are contributed by males carrying *Fs(3)Horka* are lost during the first cleavage divisions in about 20% of the XX zygotes (Szabad, unpublished data). This event produces XX//XO, female//male mosaics, so-called gynandromorphs (for a review see Janning, 1978).

Agametic gynandromorphs (without germ line cells) were generated by crossing *y v f mal; osk*^{301} *p*^{p} homozygous females with *Fs(3)Horka/TM3* males. No pole cells, which are the primordial cells for the germ line, develop in embryos produced by *osk*^{301} homozygous mothers when these are kept at 18°C (Lehmann und Nüsslein-Volhard, 1986). Eggs were collected at 18°C, and the embryos were later transferred to 25°C. In this paper, the mutation *osk*^{301} will be abbreviated as *osk*.

For gene symbols see Lindsley and Zimm, 1985; 1990.

Analysis of terminalia (analia and genitalia)

The terminalia of a sample of the regular and agametic gynandromorphs were mounted and inspected under a compound microscope. The size of the various sexually dimorphic structures was determined and expressed as a fraction of an entire intact structure. This fraction was then plotted in a diagram (for further details see Nöthiger et al., 1977).

Analysis of gonads

Gynandromorphs were aged 2-7 days and were then dissected for analysis of the gonads. For both regular and agametic gynandromorphs, the amount of gonadal tissue was expressed as a fraction of the size of non-mosaic ovaries or testes. In regular gynandromorphs, the number of ovarioles was counted and taken as a measure of the amount of ovarian soma; the amount of testicular soma was also estimated.

The gonads of agametic gynandromorphs were mounted on a slide in 45% acetic acid. To ensure a constant thickness of the mounted tissue, two thin aluminium stripes were put between the slide and the coverslip. Following removal of excess liquid, camera lucida drawings of the gonads were made and the area of the gonads was determined by planimetry. For ovarian tissue, the ovarioles were also counted. Gonads were only classified as mosaic when they were composed of both ovarian and testicular tissue.

Fate mapping of the gonadal soma

The site of the progenitor cells for the gonadal soma was mapped relative to tergites and sternites. For fate mapping, 123 regular and 128 agametic gynandromorphs were randomly selected and analyzed with a compound microscope. Hemitergites, hemisternites and gonads were separately classified for left and right primordia as XX, XO or mosaic (for details of the mapping procedure, see Janning, 1978; Merriam, 1978). Construction of the fate maps was done with a computer program kindly provided by W. Janning of the University of Münster in Germany.

Results

Mosaic gonads suggest the existence of a single primordium

Mosaic gonads were found in both types of gynandromorphs, regular and agametic (see Materials and methods). In both types, the ovarian fragments were composed of ovarioles, and the testicular fragments were usually covered with yellow coat cells. Female and male parts formed coherent areas and were not intermingled, and the ovarian and the testicular fragments usually occurred in close association.

In 306 regular gynandromorphs, 71 gonads (11.6%) were mosaic (Table 1). The ovarian fragments contained fewer ovarioles than ovaries of normal sibling females, in general less than 10 (compared to 17.5±1.9 for normal ovaries, n=32). Even in non-mosaic ovaries, the number of ovarioles varied from 12 to 21. Gonads that consisted of 10 or fewer ovarioles, however, always contained some testicular tissue.

Mature sperm or eggs develop in the gonads whenever the sex of the germ line and of the gonadal soma is the same (van Deusen, 1976; Schüpbach, 1982, 1985; Steinmann-Zwicky et al., 1989). In these cases, the gonads become large. Germ cells, however, whose sex differs from that of the gonadal soma undergo abortive development and, as a consequence, the gonad remains small (Steinmann-Zwicky et al., 1989; Nöthiger et al., 1989). This poses a problem for the analysis of regular gynandromorphs. In such animals, the somatic cells of the gonad may be in contact with germ cells of identical or of opposite sex. Thus, the amount of gonadal somatic tissue can be reliably estimated for ovarian tissue by counting the number of ovarioles, but not for testicular tissue in which no such units exist.

To avoid the disturbing effect of the germ line cells, we produced agametic gynandromorphs. The gonads of such animals develop normally, except that they are smaller (Geigy, 1931; Aboim, 1945). In 196 agametic gynandromorphs, 31 gonads (7.9%) were mosaic. Ovaries of non-mosaic control females (siblings) were composed of 13.8±2.8 ovarioles (n=81), ranging from 8 to 20. In gynandromorphs, when the number of ovarioles was 6 or below, a region of testicular tissue was always present.

In agametic flies, the amount of ovarian tissue, as measured by planimetry (see Materials and methods), turned out to be directly proportional to the number of ovarioles (Fig. 1). This observation indicates that camera lucida drawing and planimetry of ovarian and,

Table 1. *The status of gonads in regular and agametic gynandromorphs*

Types of gynandromorphs	Gynandromorphs analyzed	Gonads of gynandromorphs									
		Two ovaries	Two testes	One ovary and one testis	One ovary and one mosaic gonad	One testis and one mosaic gonad	Both gonads mosaic	One ovary present, one gonad missing	One testis present, one gonad missing	One mosaic gonad, and one missing	Both gonads missing
Regular	306	124	92	23	24	21	12	4	4	2	0
Agametic	196	120	33	8	8	7	8	4	2	0	6

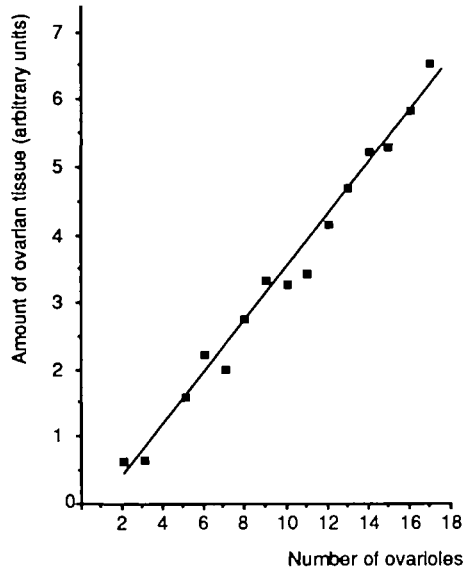

Fig. 1. The linear relation between the number of ovarioles and the amount of ovarian tissue as measured by planimetry (see Materials and methods). The data are based on 31 mosaic gonads of agametic gynandromorphs and on 76 ovaries of their non-mosaic sisters. Each symbol represents an average value of 2-11 gonads. The regression coefficient of $r=0.98$ documents the linear relation.

by inference, of testicular tissue, is a reliable way to quantify the amount present in mosaic gonads.

The results obtained for 82 mosaic gonads, 51 from regular and 31 from agametic gynandromorphs, are shown in Fig. 2A. To facilitate their interpretation, we will compare them with data for mosaic analia (Fig. 2B) and mosaic genitalia (Fig. 2C). We determined the sex and size of the analia and genitalia of 123 regular and 128 agametic gynandromorphs. In Fig. 2B, all gynandromorphs except one fall on the diagonal (shaded), reflecting the fact that the fractions of male and of female analia added up to one unit of analia. This is expected when a single primordium can give rise to either female or male analia, depending on whether it is populated by XX cells or by XO cells. In contrast, the distribution of gynandromorphs is very different for the genitalia (Fig. 2C). These data indicate the existence of two separate primordia, one for female genitalia and one for male genitalia. The female genital primordium, presumably located in abdominal segment A8, will only develop when populated by XX cells; and the male genital primordium, presumably located in A9, will only develop when populated by XO cells (Nöthiger et al., 1977; Schüpbach et al., 1978). Thus, mosaic genital structures may contain any fraction of male genitalia combined with any fraction of female genitalia; in extreme cases, a complete female plus a complete male set of genitalia, or neither female nor male genitalia, may be found. The outcome will depend on whether the respective primordium was only partially or entirely populated by XX or by XO cells. Two different primordia were also postulated for the foci controlling sexual behavior (Szabad and Fajszi, 1982).

In mosaic gonads, the amount of female and the amount of male gonadal soma add up to roughly one unit, although a number of cases are found above and below the diagonal (Fig. 2A). The sizes of gonads vary even in control flies and, furthermore, are more difficult to measure than in the analia and genitalia. These two factors are probably responsible for many of the cases deviating from the diagonal. When we count the cases that fall onto the diagonal (shaded), these numbers are 45/82 for the gonads, but only 15/56 for the genitalia. On the other hand, only 14/82 gonads form slightly more than one unit, whereas almost half of the gynandromorphs (25/56) form more than one set of genitalia. Of particular importance is the fact that we never observed a complete ovary and a complete testis on the same side of any of 424 gynandromorphs with mosaic abdomina. In contrast, two flies were found with a complete set of male and a complete set of female genitalia (Fig. 2C, upper right corner). Thus, we

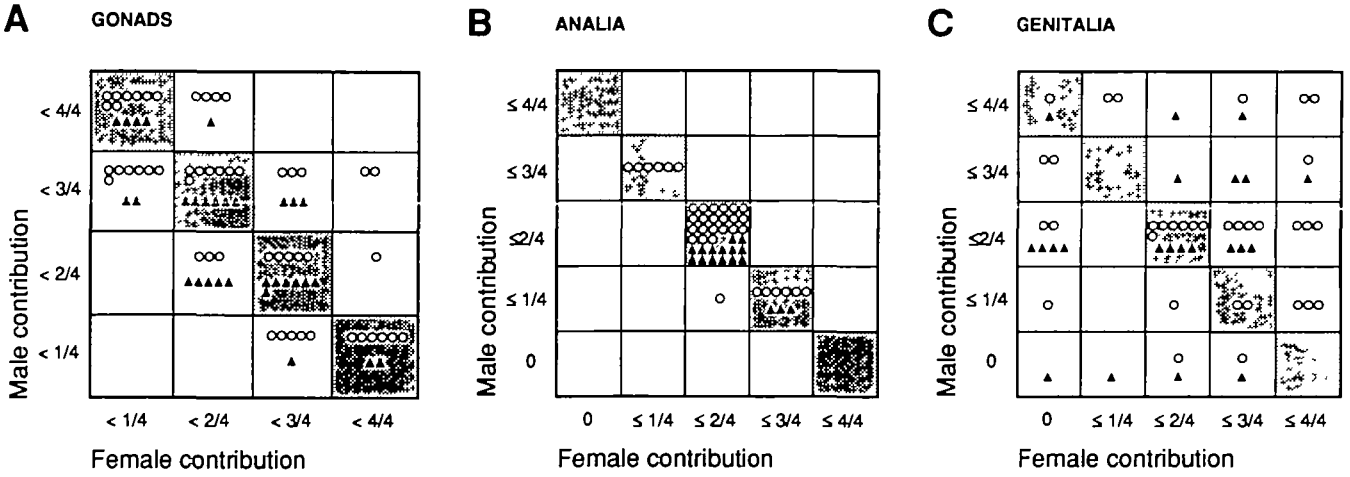

Fig. 2. The contribution of female and male tissue in gonads (A), analia (B), and genitalia (C) of regular (○) and agametic (▲) gynandromorphs. Each symbol in the graphs (○ or ▲) represents one gonad (in A) or one gynandromorph (in B and C). Ordinate and abscissa give the fraction of an entire male or female structure, respectively. In C, the two flies in the upper right square had a complete set of female and a complete set of male genitalia.

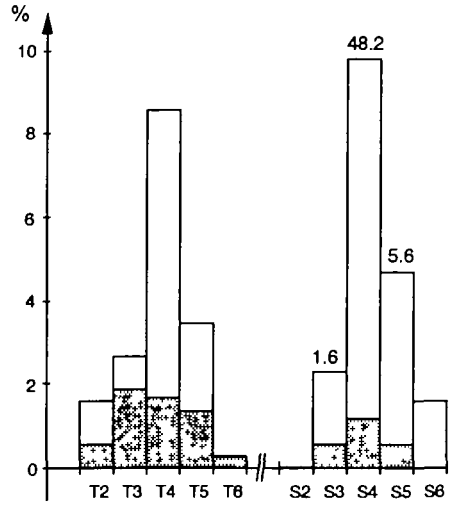

Fig. 3. Distribution of abdominal defects in 219 regular (shaded bars) and 128 agametic gynandromorphs (open bars). Ordinate gives frequency (%) of a hemitergite (T) or hemisternite (S) being absent or only reduced in size. Numbers above S3, S4, S5 give the frequency (%) with which the gonad is simultaneously absent when a hemisternite is absent or reduced on the same side.

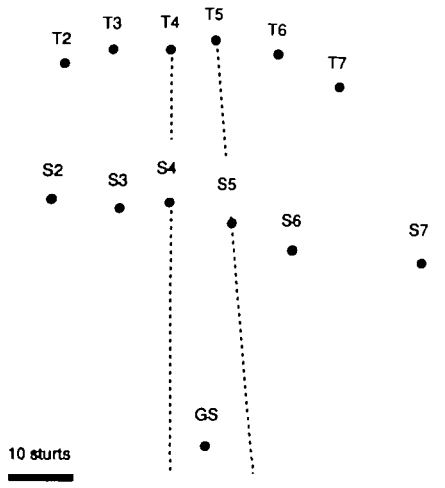

Fig. 4. Fate map of the gonadal soma (GS), hemitergites (T), and hemisternites (S). The map is based on 123 regular plus 128 agametic gynandromorphs analyzed with a compound microscope.

interpret the results presented in Fig. 2A as indicative of a single gonadal primordium whose cells give rise either to an ovary when they are XX, or to a testis when they are XO.

A substantial number of mosaic gonads (23/82) contained less tissue than one unit (Fig. 2A), and some were lacking gonads entirely (Table 1). These cases could be taken to reflect two separate primordia for male and female gonads, both having been populated, in part or entirely, by cells of the wrong genotype. Several arguments, however, speak against this interpretation. First, as we just pointed out above, some variation in sizes and some inaccuracy in measuring are inherent in our analysis of gonads. More important is the observation that both the regular and the agametic gynandromorphs exhibit defects in several other structures that are not sexually dimorphic, such as tergites and sternites (Fig. 3). Complete absence of hemitergite 4 was noticed 5 times among 438 inspected half sides of regular gynandromorphs; and 5 gonads were also absent in these same flies. The number of abdominal defects was much higher in the group of 196 agametic gynandromorphs, and so was the frequency of missing gonads. This frequency (18/392 gonads) is significantly higher than that observed for regular gynandromorphs ($P<0.05$). Regular and agametic gynandromorphs differ by the presence of the *osk* mutation which is known to interfere with abdominal development (Lehmann and Nüsslein-Volhard, 1986). We thus conclude that not only the defects in the tergites and sternites, but also the absence and defects of gonads, are consequences of the mutation *osk*. The defects in agamatic gynandromorphs, and also those in the regular gynandromorphs, are not always complete, so that structures such as tergites and gonads may only be reduced in size rather than completely eliminated.

Fate mapping and size of the gonadal soma

The fate maps derived from regular and agametic gynandromorphs were practically indistinguishable, and thus the data were combined to produce the map shown in Fig. 4. Our data place the gonadal soma into the region of the 4th and 5th abdominal segments, ventrally to the sternites in the presumptive mesoderm of the blastoderm embryo.

The progenitor cells of the left and right gonadal soma must lie relatively close to each other. This conclusion is based on three observations: First, among 480 gynandromorphs, we found only 31 cases (6.5%) in which the XX//XO border fell between the left and right gonadal primordium, as compared to some 30% for hemitergites and -sternites (Table 2). Second, both gonads were simultaneously mosaic in 20 of 80 gynandromorphs with mosaic gonads (Table 1). Finally, both gonads were missing in 6 of the 12 agametic gynandromorphs in which one or both gonads did not develop. If the events were independent, only one such animal was expected (Table 2).

The size of a primordium can be estimated from the frequency with which it is mosaic. This frequency was not significantly different for hemitergites 3, 4 or 5 and for gonads of regular and agametic gynandromorphs (Table 2). A hemitergite 4 derives from 8 to 10 precursor cells (Merriam, 1978). Assuming a similar shape of the primordia for hemitergites and gonads, the area from which the precursors of the gonads originate must comprise some 10 blastoderm cells on each side.

Discussion

Previous analyses (Sonnenblick, 1941, 1950; Aboim, 1945; Campos-Ortega and Hartenstein, 1985; Technau

Table 2. *Relationship between left and right primordia*

	A Left//right separation†			B Mosaicism						C Absence				
					structures						structures			
	Number of gynandro-morphs	male/female		Number of gynandro-morphs	formed	mosaic		both mosaic obs.	both mosaic exp.	Number of gynandro-morphs	absent		both absent obs.	both absent exp.
		n	%		*n*	*n*	%	*n*	*n*		*n*	%	*n*	*n*
Gonads	480‡	31	6.5	502	976	102	10.5	20*	5.2	196§	18	4.6	6*	0.4
T4	240‡	66	27.5	251	491	48	9.8	1	2.4	128§	6	2.3	0	0.1
S3	250‡	79	31.6	251	501	50	10.0	2	2.5	–				
S4	–			–						128§	11	4.3	1	0.2

†only those cases were considered in which one structure (gonad, T4, S3) was entirely male, the other entirely female.
‡agametic plus regular gynandromorphs. For gonads, all flies with two gonads were considered; for T4 and S3, only those flies that were mounted for microscopical inspection and had left and right structures were considered.
§agametic gynandromorphs only.
T4=hemitergite 4; S3, S4=hemisternites 3, 4.
n=number.
obs.=observed; exp.=expected.
*=significantly higher than expected (Kastenbaum and Bowman, 1970).

and Campos-Ortega, 1986; Hay et al., 1988; Lasko and Ashburner, 1990) had shown that the pole cells, after arriving in the region of the dorsolateral mesoderm, become bilaterally arranged in two longitudinal stripes, ranging from abdominal segment A5 to A7/A8 on each side of the stage 12 embryo. Later, at stage 14, they form two spherical groups of cells that are now located at the level of A5. At this stage, they are surrounded by mesodermal cells which will later form the somatic part of the gonad.

1. *The gonadal soma appears to originate from a single primordium common to both sexes*

This conclusion derives from the data in Fig. 2. These suggest that the somatic cells of testis and ovary are homologous in the sense that they originate from the same primordium. Nevertheless, the cells assume very different functions in the two sexes, as a consequence of differential activity of the sex-determining genes. Once the sex-specific cell type is formed, ovarian or testicular epithelium, the cells cannot revert and perform functions of the opposite sex, even when the activity of the sex-determining genes is switched from the female-determining mode to the male-determining mode, or vice versa (Bownes et al., 1990).

2. *Origin and size of the gonadal primordium*

Although the primitive embryonic gonad, once it is formed in the stage 15 embryo, is located at the level of abdominal segment A5, the constituent pole cells were gathered there from a more extended stripe ranging from A5 to A7/8. It is therefore possible that the mesodermal precursor cells are also recruited from the area of this stripe, not just from one segment or parasegment (Martinez-Arias and Lawrence, 1985). Two earlier studies in fact concluded that the somatic parts of the gonad derive from a large mesodermal area.

The first of these (Gehring et al., 1976) located the somatic primordium of the gonads more posteriorly and also found, at least in some samples, a much higher frequency of mosaicism, namely 19 to 37%, compared to 10.5% in our study. For a circular primordium, a two to almost four times higher frequency of mosaicism means 4 to 16 times more precursor cells, i.e. some 40 to 160 cells. This value surpasses the number of 27 to 37 mesodermal cells counted by Sonnenblick (1941) for the gonadal primordium of a 14 hour old embryo and is almost certainly too high. The reason for the discrepancy between our data and those of Gehring et al. (1976) may be that they mistook some yellow-colored *vasa deferentia*, which are derivatives of the genital disc, as parts of a mosaic testis. Stern and Hadorn (1939) have shown that yellow epithelial testis cells can migrate over the *vasa deferentia* from one side to the other. If these cases are counted as mosaic gonads, this would artificially increase the frequency of mosaicism and, in a fate map, would also move the gonadal anlage closer to the more posteriorly located genitalia.

In the second study, Lawrence and Johnston (1986) produced chimeric flies by injecting genetically marked nuclei into embryos of early cleavage stages. They found 15 females with marked patches in their ovaries, accompanied by marked cells anywhere in abdominal segments A3 to A8. Although the patches were usually coherent, this fact does not prove that the precursors of the ovarian soma are recruited from a large region spanning several abdominal segments. It is equally possible, or even more likely, that the transplanted nuclei have spread from the site of injection anteriorly and posteriorly.

The computer program we used to calculate the fate map (Fig. 4) identifies only the center of a primordium. Its size, or rather the area from which it derives, can be estimated from the frequency with which it is mosaic. This frequency (*f*) is related to the size and shape of a primordium (Hotta and Benzer, 1973; Wieschaus and Gehring, 1976). In our experiments, gonads were mosaic in 10.5% of the cases. If the primordium of the gonadal soma has a circular shape, its diameter *d* is

Table 3. *Frequency of mosaicism in hemitergites*

hemitergites	% mosaic
T3	10.6
T4	9.8
T5	11.6
T3+T4	20.2
T4+T5	19.0
T3+T4+T5	27.8

The data derive from the analysis of 123 regular plus 128 agametic gynandromorphs. There was no difference between the two groups.

equal to $2f\pi$, i.e. 6-7 sturts. This value is below the 8-12 sturts measured between adjacent abdominal segments (Janning, 1978).

Another argument for the origin of the gonadal soma from a single segment is presented in Table 3 which shows that the frequencies of mosaicism for hemitergites T3, T4 and T5 are roughly additive and proportional to the area under consideration. If the precursors for the gonadal soma were drafted from more than one segment, we should have obtained a significantly higher frequency of mosaicism, similar to the one for two or three hemitergites.

Our data do not permit us to identify the exact segment or parasegment (PS) within which the gonad is formed although the fate map points to A4 or A5. More evidence suggesting A4 or A5 as the segment from which the gonad originates is given in Fig. 3. We found a correlation between deficient hemisternites and absence of gonads. This correlation is very strong for S4, but only weak for S5 and even weaker for S3.

Another hint comes from animals mutant for *iab-4*. In such flies, abdominal tergite T4 is homeotically transformed into T3, and gonads are missing (Karch et al., 1985). The normal function of *iab-4* appears to be to regulate the homeotic gene *abd-A* in PS 9 in the ectoderm and probably in PS 10 or 11 in the mesoderm (Tremml and Bienz, 1989).

In conclusion, our data indicate that the gonadal anlage derives from a single (para)segment, and other evidence suggests that this is PS 10 or 11.

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