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Brista: a gene involved in the specification and differentiation of distal cephalic and thoracic structures in *Drosophila melanogaster*

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Summary. A gene *Brista* has been identified in chromosome 2R, in the region 60D11-E4, in which mutations cause homoeotic transformation of distal antennal structures to distal leg derivatives, and in which certain alleles also lead to upsets in the formation of distal elements of the legs. This gene is haploinsufficient for the homoeotic phenotype. Several putative null and two hypomorphic alleles have been recovered. The effects of exposure to the non-permissive temperature of a temperature-sensitive allele are cummulative and depend upon the length of the exposure during the period of antennal cell proliferation. It is suggested that this gene contributes to the stability of the state of determination in distal domain of the antennal and leg discs, and its relationship to other genes with similar mutant phenotype is discussed.

Key words: *Drosophila* – Homoetic – Mutational analysis – Transformation – Distal disc structures

Introduction

Extensive investigations pioneered by Lewis (1963, 1964) and Garcia-Bellido (1977) have now shown that the segmental and compartment fate of many groups of cells in Drosophila follows the specific derepression of genes within the Bithorax-Complex (Lewis 1978) and the Antennapedia Complex (Kaufman et al. 1980), during early embryogenesis. These functions are reflected as cell heritable fate restrictions. A number of other genes has been identified as essential for the correct topological expression of these two complexes (Struhl 1981a, 1983; Struhl and Akam 1985; Ingham 1985), some of which function only during embryogenesis (Struhl and Brower 1982), whilst other are necessary within the proliferating cells of imaginal discs and histoblast nests during post embryonic stages (Ingham 1985). There is considerable support for the idea that these genes function combinatorially in the specification of the correct developmental destination of cells (Struhl 1982a). It is within this context that we have defined and described a gene (*Brista, Ba*) required within cells of parts of the antennal disc for the correct elaboration of the arista by these cells. When this gene fails or functions inappropriately, the arista is replaced by a tarsus and distal structures of the thoracic legs fail to form correctly. These phenotypes are paralleled by alterations in the cuticle of the first instar larva of homozygotes for some of the *Brista* mutant alleles.

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Material and methods

Culture conditions

Flies were reared at 25° C, unless otherwise stated, on a standard agar, sugar, maize meal and yeast medium.

Quantification of the phenotypes

Embryonic and larval lethal combinations. The phenotype of lethal combination of *Ba* alleles was studied by observing the cuticle formed in late embryogenesis. Embryos from the required cross were collected over 8 h and allowed to develop for a further 20 h. Eggs which did not hatch were dechorinated by hand and devitellinised, cleared in acetic acid and 95% ethanol (9:1) for 15 min at 60° C and mounted in Hoyer's medium (Van der Meer 1977).

Adult phenotypes. Transformation from antenna to leg was measured in terms of *penetrance* (LP) and *expressivity* (Ex). Because Ex was found to vary continuously we used the measure (*total length of transformed tissue/total length of appendage*) × 100. The measurements were taken of one antenna of each individual using an eye-piece micrometer at X100 under a dissecting microscope, and a mean expressivity was calculated for each group. *The leg-deletion* phenotype (missing distal segments) was quantified as *penetrance* (LP). For most experiments one side of at least 100 flies was scored for each genotype under the dissecting microscope.

Temperature shifts

The temperature sensitive allele Ba^1 was used for temperature shifts experiments. This allele shows no dominant antenna-leg transformation at 29° C but strong effects at 19° C. Eggs were collected for 8 h periods at 25° C and cultured in food vials at 29° C, except for a low temperature pulse of 48 h duration at 19° C. The TSP of the leg-deletion phenotype was determined by temperature shifts, from 19 to 29° C and from 29 to 19° C of groups of individuals from eggs collected at 25° C and grown at either tempera-

ture until the shift. The genotype used for this experiment was $Dp(Y;2)Kr^+$; Ba^1/Ba^1.

Genetic variants

All variants are listed in Lindsley and Grell (1968) except for the following. Ba^1 was recovered following gamma-irradiation and selected because of its dominant antenna-leg transformation by J.R.S. Whittle and kept as the heterozygote stocks *cn bw sp* $Ba^1/SM5$ and $BlBa^1/SM5$. The mutations *Art*2 and *Art*3 were provided by James Kennison and the recessive allele Ba^M was provided by Sato (1984). $T(2;3)Pc^{3+RAntp}$ (Ba^j) was found by Gerd Jürgens in a reversion experiment with Pc^3. This translocation contains a breakpoint at the junction of 60DE. Ba^5 was found by Stanley Tiong after EMS mutagenesis. Full details of these mutations are set out in Table 1. $Dp(Y;2)Kr^+$ and $Dp(Y;2)Kr^+sp^+$, are insertions of regions 58A–B; 60A–B; 60D–F5 and 60A–B; 60F5 respectively into the Y-chromosome, and were provided by Nüsslein-Volhard but characterized by the authors (Sunkel 1983). We also defined further the breakpoints of the ring Y-chromosome $Dp(Y;2)bw^+Y^c$ as involving 59D–60E3,6. A number of deletions of distal 2R were used to define the exact location of *Brista*. They include; $Df(2R)bw$[58D5–6;59E4–F1], $Df(2R)P\times$[60B–D1], $Df(2R)P\times{}^2$[60C6–D11], $Df(2R)M$–C^{33a}[60E2–3;60E11–12], $Df(2R)Kr^-$[60F1–F5] and $T(1;2)sc^{S2}$[60E6–8;60F5].

Mutagenesis

Two different mutagenesis experiments were carried out. In the first we selected for the increased expression of the dominant antenna-leg homoeotic phenotype in the genotype $Dp(Y;2)Kr^+sp^+;Ba^1/cn\ bw\ sp^*$, where * represents the mutagenized chromosome. The selection scheme was based on the observation that $Dp(Y;2)Kr^+sp^+;Ba^1/Ba^+$ shows no transformation while $Dp(Y;2)Kr^+sp^+;Ba^1/Ba^1$ shows strong antenna-leg transformation. Males of the genotype $Dp(Y;2)Kr^+sp^+;cn\ bw\ sp$ were fed EMS (Lewis and Bacher 1968) and crossed to females *cn bw sp* $Ba^1/Sm5$. White eyed male progeny carrying the duplication were examined for antennal transformation. Two putative alleles of Ba^1 were recovered from a total of 13593 chromosomes. The first one, Ba^2 showed a dominant antenna-leg homoeotic and also the leg deletion phenotype. Ba^2 was homozygous lethal and did not complement Ba^1 but unfortunately was lost after considerable study.

The second putative mutation was designated *Ba*4. This mutant was lethal as a homozygote and did not complement either Ba^1 or Ba^2. $Ba4/Ba^+$ individuals eclose at very low frequency and show several phenotypic effects, a) *Minute* delay and thin bristles like that of $M(2)C^{33a}$, b) irregular eye facets like *IF*, c) 85% of individuals show complete loss of one or more legs like Kr/Kr^+ and d) all individuals show a slight antenna-leg homoeotic transformation. Analysis of salivary gland chromosomes of these two putative mutations revealed that Ba^2 was cytologically normal but that *Ba*4 was associated with a large deletion of the region 60D1,5–60F5 (Sunkel 1983).

In the second mutagenesis screen, homozygous wild-type males carrying a lethal-free chromosome marked with *bw* were fed EMS (Lewis and Bacher 1968) and crossed to *Bl sp*/*SM5* females. Individual *bw*/*SM5* or *bw*/*Bl sp* males were crossed to *cn bw sp* $Ba^1/SM5$ females to test their ability to complement Ba^1. One putative allele of Ba^1 was recovered in 439 chromosomes tested. Ba^3 was selected because in combination with Ba^1 it caused the flies to die as pharate adults showing extreme malformation of the antenna and the legs.

Results

We firstly present detailed description of a number of mutations, following this with the evidence that they all belong within a single complementation group which we have called *Brista*.

The phenotype of Brista mutations

Mutations at the locus *Ba* cause alterations in the antenna and the thorax of the adult cuticle but phenotypic effects vary with the mutant allele. Some heterozygous combinations of alleles cause lethality during late embryogenesis or early in the first larval instar (data not shown). These individuals show varying degrees of abnormalities in the larval cuticle.

Adult phenotypes. Ba^1, Ba^2, *Ba*4 and Ba^5 heterozygotes all show a dominant phenotype of antenna to leg transformation. The distal segments of the antenna are replaced by corresponding segments of the mesothoracic leg. Figure 1 shows a range of transformations of Ba^1 heterozygotes which have been cultured at different temperatures. At the permissive temperature (29° C) the antenna is completely normal (Fig. 1a). At 25° C the base and part of the arista bear an irregular number of bracted bristles and these sometimes extend into the third antennal segment (Fig. 1b and 1c). When the individuals are grown at the non-permissive temperature (19° C), the whole arista bears bracted bristles and an occasional distal complete claw organ (Fig. 1d). The arrangements of the bracted bristles in the transformed tarsus follows the same patterns as those found in the mesothoracic tarsus; two large black and blunt bracted bristles are located proximally and distally in all but the most distal tarsal segment. In none of the flies heterozygous for these mutant alleles did the transformation extend more proximally than the distal part of the third antennal segment.

Although most mutations described in this report are dominant with respect to the antennal homoeotic transformation, two recessive mutations (Ba^3 and Ba^M) have been isolated. Ba^3 homozygotes die as pharate adults, in which the most distal part of the arista is sometimes present but never show evidence of the third antennal segment. The second segment shows a large number of ectopic bristles some of which are bracted (Fig. 2).

The antenna is the only site of homoeotic transformation but in several combinations of *Ba* alleles the thoracic legs show abnormal cuticular patterns, in particular the loss of distal segments (the leg-deletion phenotype). This phenotype was first observed in Ba^2/Ba^+ flies where the most distal tarsal segment was missing and the remaining segments slightly shorter. Later the leg-deletion phenotype was found in other allelic combination like $Dp(Y;2)Kr^+sp^+$; Ba^1/Ba^1 when grown at the non-permissive temperature (Fig. 3a). A somewhat stronger leg-deletion phenotype was observed in the genotype $Dp(2R)bw^+Y^c;Ba^1/Ba^1$ shown in Fig. 3b. All legs of these flies show a constant phenotype

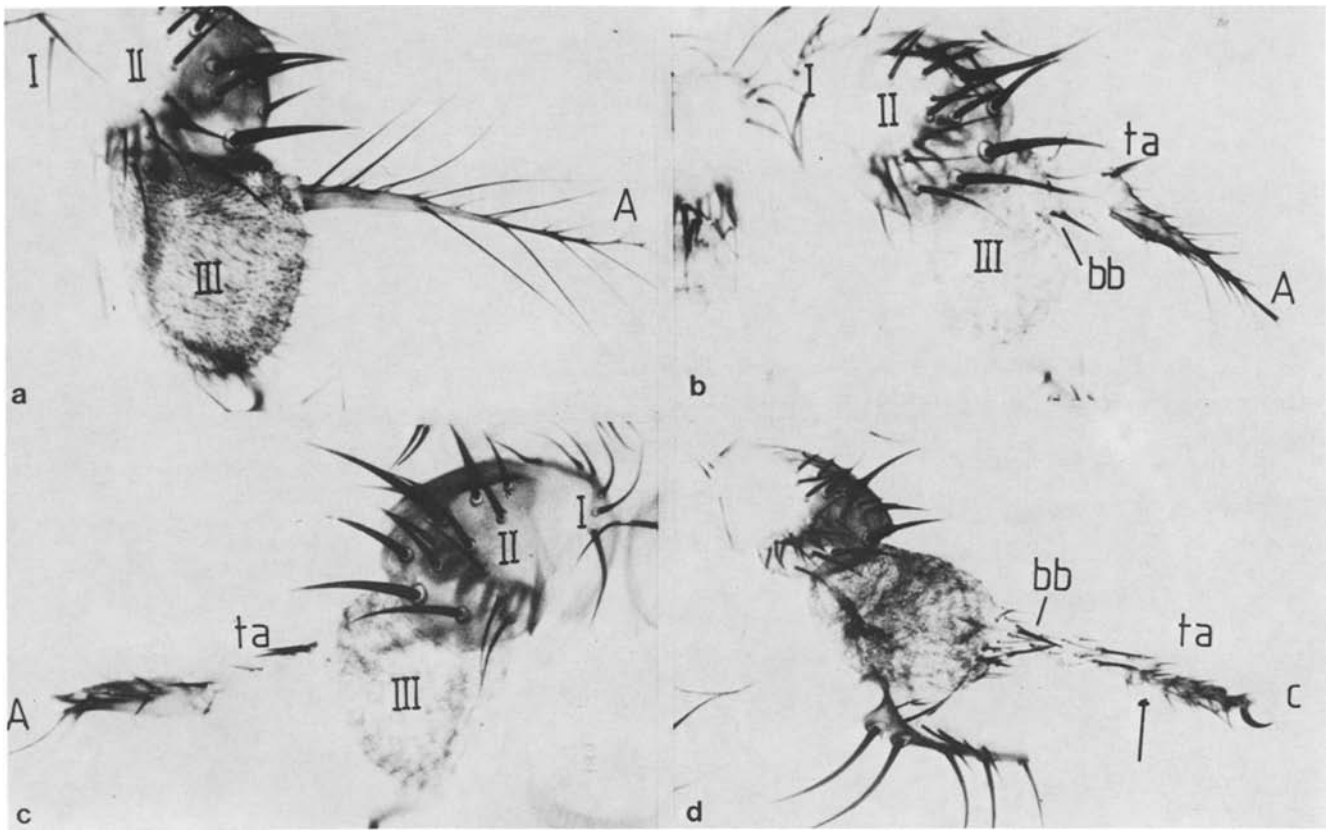

Fig. 1a–d. Homoeotic transformation of the antenna in Ba^1/Ba^+ cultured at three different temperatures. **a** After growth at 29° C the antenna is almost normal showing the arista (*A*), a slight swelling of the aristal base and the normal antennal segments (*I, II, III*). **b** and **c** show the antenna of flies grown at 25° C with normal I and II segments and bracted bristles in the III segment. The proximal arista is transformed to tarsus and the distal antenna is normal. **d** Shows the antenna of an individual grown at 19° C where the I and II segments are normal but the III segment bears bracted bristles. The arista is transformed showing partial segmentation of the cephalic tarsus (*arrow*) and has a claw. *bb* = bracted bristles, *ta* = tarsus

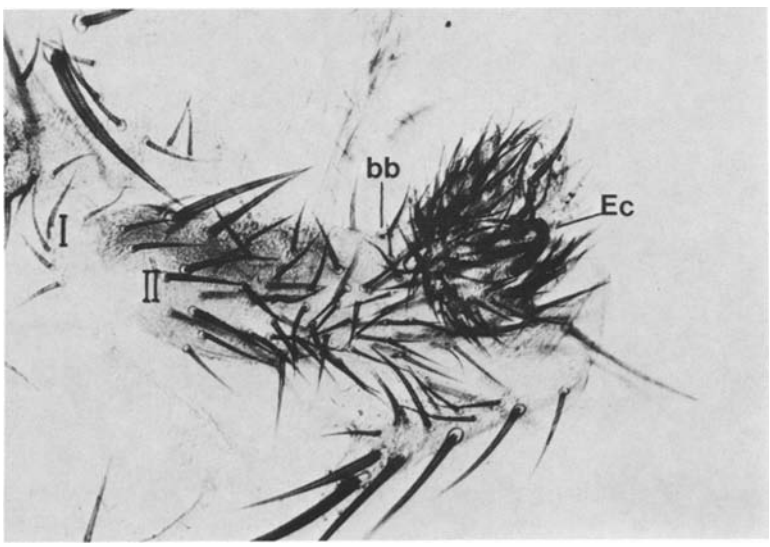

Fig. 2. Antennal abnormalities found in Ba^3/Ba^3 pharate adults. The antenna of these individuals show a normal *I* segment, however the *II* segment is enlarged and some of the bristles bear bracts. Large ectopic bristles can also be seen distally. There seem to be no remains of antennal segment *III*. Ec = ectopic bristles

of abnormal femur and tibia morphology with absence of bracts and no distal tarsal structures. The $Dp(2R)bw^+Y^c$ does not complement fully the *Brista* locus since these flies die as pharate adults. Other mutant alleles like Ba^3 when homozygous also cause severe leg defects (Fig. 3c). The legs are distorted and lack all elements distal to the tibia. Duplications are sometimes seen in the femur which bears a large number of ectopic bristles. None of the ectopic bristles found are bracted even though normal legs bear bracted bristles in the femur.

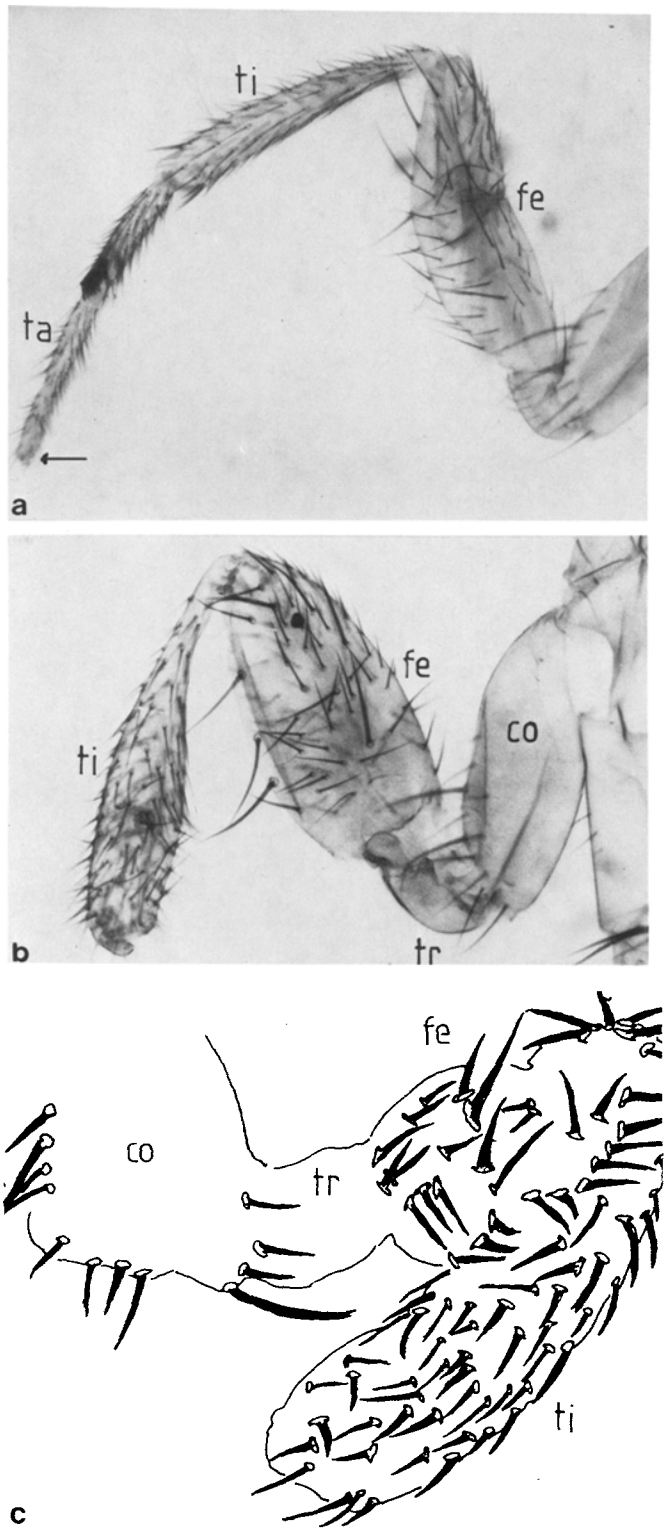

Fig. 3a–c. Distal leg abnormalities present in phare adults of different genetic combinations of *Ba* mutations. **a** The leg-deletion phenotype found in pharate adults of the genotype $Dp(Y;2)Kr^{+}sp^{+}$; Ba^{1}/Ba^{1} grown at the non-permissive temperature (19° C). The leg is mostly normal except for the loss of the most distal element including the claw. Also note that neither the basitarsus nor any of the more distal tarsal elements show proper segmentation. **b** Leg-deletion phenotype found in individuals of the genotype $Dp(2R)bw^{+}Y^{c};Ba^{1}/Ba^{1}$. While the coxa and trochanter are normal, the femur is enlarged. The tibia is also abnormal in overhall shape showing disorganized bristles and swelling of the distal end. No elements are found distally. *co*=coxa, *fe*=femur, *ti*=tibia. **c** The leg-deletion phenotype found in Ba^{3}/Ba^{3} pharate adults. The coxa and trochanter are normal but both the femur and tibia show very disorganized pattern of bristles as well as abnormal shape. No elements are found distally

Larval phenotype. All the alleles which have dominant effects are recessive lethal and cause arrest during late embryogenesis and have cuticular abnormalities, except for Ba^{1} homozygotes which die during the first instar without any morphological abnormality. The most consistent phenotypic changes are in the head and thorax of the embryo. In the head (Fig. 4a, b) the antennal and labial sense organs, the "H" piece and the maxillary sense organ are absent. In the thorax, all three pairs of Keilin's organs are missing (Fig. 4c).

Evidence for a single gene function from genetic complementation and mapping

The homoeotic effect of Ba^{1} was mapped using the markers *cn, bw* and *sp* to 0.8 cMs distal to *sp* in chromosome 2R. Recombinants were backcrossed to a *cn bw sp* Ba^{+} stock and their progeny grown at 19° C to maximise expression of the homoeotic phenotype.

Ba^{1} survived in heterozygous combinations with most deficiencies tested (see materials and methods, data not shown) except for *Df(2R)Ba4*. The only interval distal to *sp* which is not uncovered by any of these deficiencies except the latter, is 60D11–E4 and as none of these deletions exhibits the homoeotic phenotype shown by the large deletion *Df(2R)Ba4* (see materials and methods) it is likely that both the recessive lethality as well as the homoeotic phenotype map within the interval 60D11–E4.

The question remained as to whether the homoeotic and leg-deletion phenotype and the recessive lethality map to the same site in this interval. This problem was examined by complementation analysis between Ba^{1} and other mutations reported which show antennal-leg transformation and map to this same region. Details of all the mutations studied are shown in Table 1 and the results of complementation tests in Table 2. The results indicate that Ba^{j}, Art^{2}, Art^{3} and Ba^{M} are alleles of Ba^{1}. Firstly, all possible combinations of Ba^{1}, Art^{2}, Art^{3} and Ba^{j} are lethal and the recessive lethality of these mutations is associated with chromosomal breaks in the 60DE region. Secondly, the recessive phenotype of Ba^{M} has also been mapped to the same region (Table 1). Furthermore, the genotype Ba^{1}/Ba^{M} has abnormalities in the antenna and the thoracic legs resembling those of Ba^{3} homozygotes and those observed in the genotype $Dp(2R)bw^{+}Y^{c};Ba^{1}/Ba^{1}$.

Variability in behaviour between different alleles of Brista

The phenotypic variability between the different alleles indicates that whilst some might be null mutations of the locus others, (Ba^{3} and Ba^{M}) are clearly not.

We have studied the phenotype of all lethal combinations of alleles and putative alleles including heterozygotes with *Df(2R)Ba4*. The results (Table 3) show that the series of alleles fall into two classes, the first in which the abnormal phenotype of the embryos hemizygous for Ba^{1}, Ba^{3} or Ba^{M} with *Df(2R)Ba4* is more extreme than the corre-

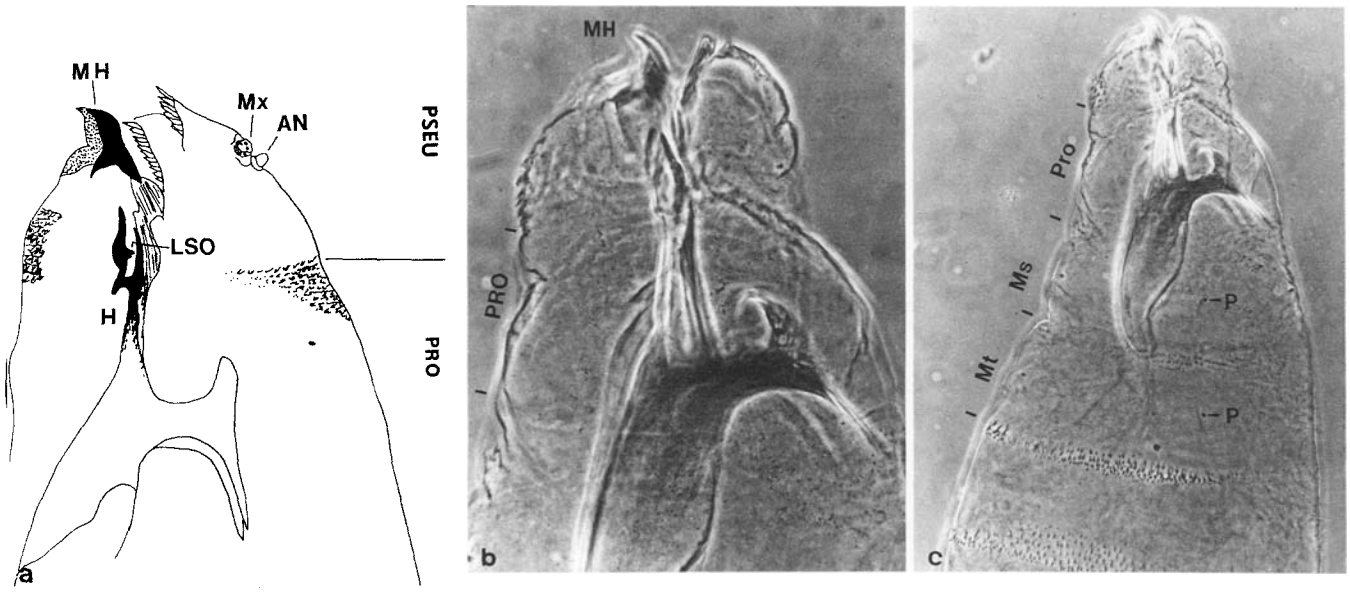

Fig. 4a–c. Larval structures affected by null mutations of the locus *Brista*. **a** Drawing of the first instar larval head of a wild type *Drosophila*. Lateral view which shows the Prothorax (*PRO*), Pseudocephalon (*PSEU*), antennal sense organ (*AN*), maxillary sense organ (*MX*), the mouth hooks (*MH*), the "H" piece (H) and the labial sense organ (*LSO*). **b** The head of a Ba^5/Ba^5 embryo. While the overhall structure of the head is normal there are a number of sensory structures missing, they include H, *LSO*, *Mx* and An. Other structures like the mouth hooks are normal. **c** Deleted structures within the thoracic cuticle of the same larva as in a. The individuals show complete absence of all six Keilin's organs. However, within the prothorax (*Pro*), mesothorax (*Ms*) and metathorax (*Mt*), the vertral pits (*P*) can be seen

Table 1. Origin and genetic data for the mutations and chromosome aberrations affecting the locus *Brista* that were used in this study

Mutation	Mutagen	Phenotypes		Mapping recombination	Cytology
		dominant	recessive		
Ba^1	gamma irradiation	A–L	Normal	2–107.8	60D11–E4
Ba^2	EMS	A–L L–D	–	–	Normal
Ba^3	EMS	Normal	A–L L–D	–	Normal
Ba4	EMS	A–L	–	–	Df60D1,5–F
Ba^5	gamma irradiation	A–L	structure missing	–	Normal
Ba^j	gamma irradiation	A–L	structure missing	–	T(2;3)60DE
Ba^M	spontaneous	Normal	A–L	2–107.6	Normal
Art^1	spontaneous	A–L	–	–	T(1;2)60DE
Art^2	gamma irradiation	A–L	structure missing	2–107.6	Normal
Art^3	gamma irradiation	A–L	–	–	In(2R)60DE

A–L = antenna-to-leg transformation. L–D = leg-deletion phenotype

Table 2. Genetic complementation tests between eight putative *Brista* alleles

		Female chromosome							
		Ba^1	Ba^3	Ba^4	Ba^5	Ba^j	Art^2	Art^3	Ba^M
Male chromosome	Ba^1	E	P	E	E	E	E	E	P
	Ba^3		P	E	P	E	P	P	P
	Ba^4			E	E	E	E	E	E
	Ba^5				E	E	E	E	P
	Baj					E	E	E	E
	Art^2						E	E	P
	Art^3							E	P
	Ba^M								V

E = embryonic lethal, P = pupal lethal and V = viable

Table 3. Larval structures in genotypes containing different *Brista* alleles

	H	K	L	A	Mx	Mh	c
Ba^1/Ba^1	+	+	+	+	+	+	+
Ba^3/Ba^3	+	+	+	+	+	+	+
Ba^M/Ba^M	+	+	+	+	+	+	+
Ba^1/Df	+−	−	+−	−	−	+	+
Ba^3/Df	+	+−	+−	+	+	+	+
$Ba^5/Ba5$	−	−	−	−	−	+	+
Ba^5/Df	−	−	−	−	−	+	+
Ba^j/Ba^j	−	−	−	−	−	+	+
Ba^j/Df	−	−	−	−	−	+	+
Ba^M/Df	+	−	+−	+−	+−	+	+
Art^2/Art^2	−	−	−	−	−	+	+
Art^2/Df	−	−	−	−	−	+	+

HE = "H" piece; K = Keilin's organ; L = Labial sense organ; A = Antennal sense organ; M = Maxillary sense organ; Mh = Mouth hooks; c = cirri. − = indicates absense of the structure. + = indicates the structure is present. +− = indicates that some animals show the structure and some do not

Table 4. Penetrance and expressivity of the homoeotic antenna-to-leg transformation and leg abnormalities in genotypes containing different *Brista* alleles

	Antenna			Leg	
	N	P	Ex	N	Lp
*Ba^1/+	320	100	94± 2	400	0
DfBa4/+	35	100	10± 8	105	0
Ba^5/+	78	100	38±11	234	0
Ba^j/+	150	98	15± 9	200	0
Art^2/+	152	100	58±12	200	0
Ba^3/Ba^3	87	−	−	261	100
Ba^M/Ba^M	122	−	−	200	100
Ba^3/Ba^1	78	−	−	234	100
Ba^3/Art^2	102	−	−	306	100
Ba^3/Ba^M	109	−	−	327	100
$Ba^3/Ba^3/Ba^+$	103	−	−	400	0
$Ba^M/Ba^M/Ba^+$	96	−	−	200	0
*$Ba^1/Ba^1/Ba^+$	118	100	68±19	400	97
*Ba^1/Df/Ba^+	158	100	41±18	400	5
$Ba^5/Ba^5/Ba^+$	71	100	31±18	213	0
Ba^5/Df/Ba^+	67	100	34±21	201	0
Art^2/Df/Ba^+	75	98	67±22	225	0

* These individuals have been grown at 19° C. In this table the only genetic combinations included are those with homoeotic transformation, abnormal antennal patterns or the leg-deletion phenotype. $Ba^+ = Dp(Y;2)Kr^+$. For N, P, Ex and Lp see material and methods

sponding homozygotes. The second class contains Art^2, Ba^5 and Ba^j and each of these heterozygous with *Df(2R)Ba4* shows the same phenotype as the corresponding homozygotes. This suggests that the alleles in the first class may be hypomorphs and those in the second class may be null mutations. However, it is important to mention that this classification is based upon their early lethal phenotypes and does not include the homoeotic or leg deletion phenotype. If these are included the situation becomes more complex since none of the 'null' alleles show leg-deletion phenotype as hemizygous while Ba^2 did clearly showed it. This

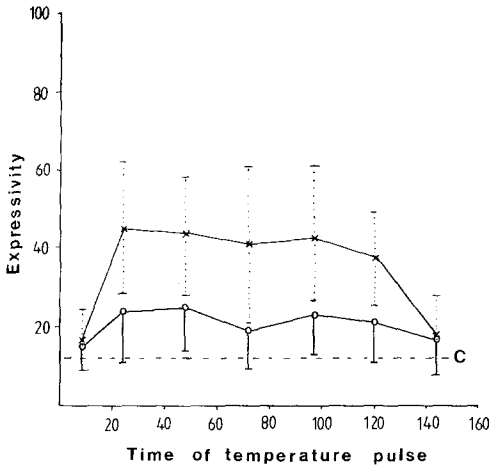

Fig. 5. Effects of exposure to a non-permissive temperature pulse of either 24 (o) or 48 (x) h upon the homoeotic phenotype in the antenna. The dotted line (*C*) indicates the level of transformation for continuous growth at 29° C. The deviation from the mean is indicated by vertical lines for each pulse. The measure of homoeotic transformation (Expressivity) is described in material and methods

could indicate that Ba^2 was indeed a null and that all other alleles are hypomorphs. One argument against this is highlited in Table 4 were we present a quantitative analysis in terms of penetrance and expressivity of the homoeotic and the leg-deletion phenotype in genotypes with different numbers of mutant and wild type copies of *Brista*. The data show that the locus is haploinsufficient with respect to the homoeotic phenotype, though the transformation is confined to the base of the arista, where a variable number of bracted bristles also appear. However, *Df(2R)Ba4* does not show the leg deletion phenotype, even though this phenotype is exposed in the genotype $Dp(2R)bw^+ Y^c; Ba^1/Ba^1$ which carries of proximal duplication.

The data also suggests that Ba^3 and Ba^M are hypomorphs because as heterozygotes these alleles do not show the haploinsufficient phenotype and because one extra copy of the Ba^+ with two copies of these alleles ($Ba^+/Ba^3/Ba^3$) suppresses the homoeotic and leg-deletion phenotype.

Temperature sensitive periods for the homoeotic and leg-deletion phenotypes

The embryonic recessive lethality of the dominant *Brista* alleles indicates an early requirement for the Ba^+ gene but does not give any clue as to when antennal and thoracic imaginal cells require the Ba^+ product.

To determine the temperature sensitive period of the homoeotic phenotype we used as stock the genotype *cn bw sp* $Ba^1/SM5$ reared at the permissive temperature (29° C). Eggs were collected over an 8 h period at 29° C. Subsequently, a single 24 h or 48 h exposure to 19° C was given to the sample after which it was cultured at 29° C. The results are shown in Fig. 5. Following any pulse given between 8 and 24 h after egg laying (AEL) and puparium formation (120 h AEL), the level of expressivity in the emerging adults is very similar. For a 24 h pulse all emerging flies show about 20% transformation and for a 48 h pulse show a 40% transformation. The latter one is significantly different from the control value (10%).

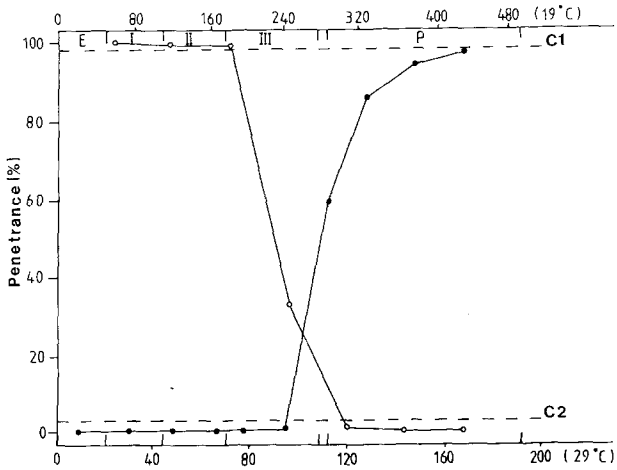

Fig. 6. The effects of temperature shifts upon the penetrance of the leg deletion phenotype. The genotype used for this experiment was $Dp(Y;2)Kr^{+}/Ba^{1}/Ba^{1}$. On the top scale growth is given in hours AEL at 19 C and at the bottom for growth at 29° C. The periods of embryogenesis (*E*), first (*I*), second (*II*), third (*III*) instar and puparium formation (*P*) are shown underneath. Open circles (o) indicate shifts from 29° C to 19° C and the closed circles (•) indicate shifts from 19° C to 29° C. The dotted lines represent controls from continuous growth at 19° C (C1) or at 29° C (C2). The results were quantified as the penetrance of the leg-deletion phenotype

The results also indicate that the level of expressivity of the homoeotic transformation depends upon the length of time spent at the non-permissive temperature rather than the precise time at which the pulse is given and the data define the temperature sensitive period (TSP) to extend from early embryogenesis until puparium formation.

We used the genotype $Dp(Y;2)Kr^{+};Ba^{1}/Ba^{1}$ to study the response of the leg-deletion phenotype to temperature change because at 19° C this genotype shows complete penetrance whilst at 29° C the penetrance is zero. The data for this temperature shifts are summarized in Fig. 6 and indicate that the TSP for the leg-deletion phenotype is sharply defined to be at the end of the third instar period.

Discussion

This paper presents genetic evidence for a gene at the distal tip of chromosome 2R in the cytogenetic region 60D11–E4 which has a role in maintaining segment identity in distal cells of the antenna and which is also required for the correct differentiation of distal elements in the legs. Failure of this gene function leads to a homoeotic transformation of the distal antenna to tarsal structures, and to loss of distal elements in thoracic legs. The locus seems also to contain functions required during early embryogenesis as a number of recessive lethals have been recovered which cause loss of cuticular and internal structures of the late embryo.

Complementation analysis based upon the antenna-leg homoeotic phenotype and upon recessive lethality have their simplest explanation in a single genetic element. Eight mutations in chromosome 2R chosen because they showed antennal-leg homoeosis ($Ba^{1,2,4,5,j}$, $Art^{1,2,3}$) share a common lethal in the vicinity of 60D11–E4. One mutation (Ba^{3}), selected because it failed to complement the lethality of Ba^{1}, itself shows antenna to leg transformation as a homozygote, and a mutation Ba^{M} identified as homozygous viable and having a homoeotic transformation in the antenna, fails to complement the other group of recessive lethal mutations. All these ten mutations, listed in Table 1, are therefore considered to be alleles of *Brista*. Chromosomal deletions localise Ba^{1} to 60D11–E4, and three of the alleles (Ba^{j}, Art^{1} and Art^{2}) have in common chromosomal aberrations in the 60DE region.

The homeotic transformation produced by *Ba* in the distal antenna is the consequence of a failure of this gene function. The deficiency (*Df(2R)Ba4*) clearly lacks gene functions to the right of *Brista* ($M(2)C^{33a}$, *Kr* and *IF*) and cause early lethality in trans with most alleles. In heterozygotes over a wild type chromosome the deletion also causes the dominant homoeotic transformation, showing that *Ba* is a haploinsufficient locus. This result also indicates that the deletion must uncover most of the locus, since a duplication of the proximal region ($Dp(2R)bw^{+}Y^{c}$) complements the recessive lethality of Ba^{1}/Ba^{1} but leaves exposed the more distal elements causing Ba^{1} to become a late pupal lethal. Ba^{5}, Ba^{j} and Art^{2} behave as null alleles; in heterozygotes the homoeotic effect of each of these alleles resembles that of the deletion heterozygote (*Df(2R)Ba4*), and the embryonic lethal phenotype for each homozygote is identical to that of the corresponding heterozygote with the deletion *Df(2R)Ba4* (Table 3).

Alleles Ba^{1}, Ba^{3} and Ba^{M} behave as hypomorphic; they exhibit abnormalities and are arrested during embryogenesis as hemizygotes (Table 3) but survive to show homoeotic transformation as pharate adults when homozygous. However, Ba^{1} is uniquely different to Ba^{3} and Ba^{M} in that the genotype $Ba^{1}/Ba^{1}/DpBa^{+}$, at the non-permissive temperature has both homoeotic transformation and the leg deletion effect (Table 4), but Ba^{3} or Ba^{M} produce wild type adults in the genotypes $DpBa^{+}/Ba^{+}/Ba^{3}$ and $DpBa^{+}/Ba^{+}/Ba^{M}$.

In thoracic legs, some alleles showed abnormalities which progressively were the loss of tarsal segments, the loss of the tibia, and in extreme cases the appearance of unbracted bristles on the femur. $Df(2R)Ba4/Ba^{+}$, the heterozygous deletion for *Brista*, had no leg abnormalities so in this respect the locus is not haploinsufficient. This phenotype only appears in genotypes which are putative null alleles or which have very low Ba^{+} activity, for example, $Ba^{+}/Ba^{1}/Ba^{1}$ grown at 19° C or Ba^{M} homozygotes. Genetic combinations like Ba^{3}/Ba^{3} or $Dp(Y;2)bw^{+}Y^{c};Ba^{1}/Ba^{1}$ might represent even lower *Brista* activity as they show abnormalities in more proximal leg segments. In contrast to the expressivity and penetrance of the homoeotic transformation which shows a wide variation between different genotypes, the leg-deletion phenotype has a clear threshold to its expression because it is either almost 100% penetrant or not detectable in any particular genotype.

The allele Ba^{1} showed temperature sensitivity in its effects upon homoeotic transformation of the antenna and for the leg abnormality. The extent of the homoeotic transformation was dependent upon the duration of the period of the cold-shock rather than on its precise timing. This result would be consistent with an extended or continuous requirement for this gene function during antennal disc cell proliferation, rather than its role as a 'trigger' for a developmental event either early or late. The temperature sensitive period for the leg deletion phenotype is sharply defined around 96 h after egg deposition, at puparium formation,

which is the period during which the growth rate of leg imaginal cells increases considerably (Postlethwait and Schneiderman 1971). It is not known whether this increase in division rate is particular to cells located distally within the disc.

It is instructive to compare the phenotypes of *Brista* mutations in larval and imaginal tissues. In the latter the domain of function appears to be restricted to distal regions of the antennal and leg discs. However, no cell-lineage restrictions separating proximal and distal elements of the antenna arise until at least the end of the third larval instar (Postlethwait and Schneiderman 1971; Morata and Lawrence 1979). This would indicate that the function of this locus is not restricted to a particular polyclone within the eye-antennal disc.

With the exception of Ba^1 homozygotes, which hatch to form first instar larvae with normal cuticular morphology, all the other homozygotes for *Brista* alleles fail to hatch and have abnormalities in cuticular structures of the head and the thorax. The head of *Drosophila* is thought to contain six segments (Jürgens et al. 1986). The structures clearly absent in these embryos derive from four head segments, the antennal, mandibular, maxillary and labial segments. There is no evidence that the other two head segments are affected.

In the thoracic segments the structures affected, the Keilin's organs, identify the site of origin of the distal appendage in each of these segments. Furthermore, it has been shown that the Keilin's organs are made up of three hairs, two derived from the anterior compartment and one derived from the posterior compartment of each segment (Struhl 1984). This would indicate that *Brista* function is not restricted to a particular compartment.

The phenotype that follows the loss or failure of the *Brista* gene further reinforces the impression that there is a close developmental link between the antenna and leg in Drosophila. The hyploinsufficient phenotype of *Brista* speaks either for the lability or the complexity of determination and differentiation of the antennal structures. *Brista* is one of many loci in which mutations produce antenna to leg transformations (Ingham 1985; Struhl 1982b; Ingham 1984; Denell 1978; Gehring 1970). Gene functions within the Antennapedia Complex (Ant-C) especially *Antennapedia* and *Sex Combs Reduced*, are expressed in thoracic or posterior gnathal anlagen and not in the segments of the head that give rise to the antenna (Martinez-Ariaz and Struhl 1986). Ectopic expression of ANT-C functions (*Antp* dominant alleles) cause antennal cells to develop leg structures, and recessive-lethal null alleles of *Antp* permit antennal structures to be elaborated in thoracic anlage (Struhl 1981b). Furthermore, it has been recently shown that when a cDNA clone from the Antennapedia complex is reintroduced into the genome under the control of a heat-shock promoter, the result of heat shock is the production of homoeotic transformation of antenna to leg (Schneuwly et al., unpublished work). Struhl (1982b) has shown that the gene *spineless-aristapedia* (ss^a) has a domain of function within part of the antennal disc, but does not have the same properties as the genes in the ANT and BX complexes which establish segment-identity within defined polyclonal lineages in the embryo. In this respect *Brista* resembles ss^a. Ingham has identified two other genes, *Trithorax* (1981) and *supersexcombs* (1984), failure in which cause antenna to leg homoeosis, and he suggests that both genes regulate the ANT and BX complexes. Mutations in the gene *Brista* cause disturbances in the formation of distal elements both of the antennal and leg discs, and it is presently equally valid to interpret this as a gene providing an essential component in the generation of distal structures in these discs, and to consider the homoeosis as a secondary consequence of the partial failure of this component in the antennal disc, which from the evidence cited above, appears to have a more liable determination state than other discs. Mutations in the gene *decapentaplegic* (Spencer et al. 1982) and *Distal into proximal* (Kerrige 1981) both interfere specifically with the formation of distal structures in several disc structures. Resolution between a primary segment-identity role and a 'distal-element' role for *Brista* will depend upon examination of the spatial and temporal distribution of its transcription and the extent of independence with the expression of genes within the Antennapedia-Complex.

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