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Interaction between *spineless-aristapedia* gene and genes from *Antennapedia* and *bithorax* complexes of *Drosophila melanogaster*

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ABSTRACT Mutations in the *spineless-aristapedia* (ss^a) gene of *Drosophila melanogaster* are pleiotropic, and their classical manifestations include a reduction in size of all bristles (spineless phenotype), transformation of distal parts of antennae into tarsal segments of the mesothoracic leg (aristapedia phenotype), and, in extreme alleles, fusion of tarsal segments on all six legs and the transformed aristaes. We isolated a new allele, which is a severe loss-of-function mutation and, in addition to the above-mentioned features, is characterized by amplification of sex combs on the first leg. This phenotype can be caused by a change in the expression of the *Sex combs reduced (Scr)* gene of the ANTP-C. Identification of this phenotype, together with observed variations in the extent of the fusion of tarsal segments in the legs of different segments, raised the possibility that ss^a interacts with homeotic genes controlling the identity of segments. This possibility was tested in genetical experiments using flies with loss-of-function mutations in several homeotic genes and flies transformed by heat shock-driven homeotic genes. Analysis of adult phenotypes of different ss^a alleles in the background of under-, over-, or ectopic expression of some genes of *BX-C* and *ANT-C* suggests that the ss^a product is required to prevent the effect of the homeotic gene products in the distal segments of the appendages.

KEY WORDS: *determination, ANT-C, BX-C, ss^a, antennae, arista, leg, tarsal segments, Drosophila melanogaster*

Introduction

Determination of cells to the formation of different appendages occurs at early stages of embryonic *Drosophila* development as a result of expression of selector genes in the specific domains along the body-axis (Cohen, 1993). However, the ectopic expression of *Antennapedia (ANT-C)* and *bithorax (BX-C)* gene complexes during the second-third instar larvae period can change the identity of one appendage to the other (Schneuwly *et al.*, 1987; Gibson and Gering, 1988; Gibson *et al.*, 1990; Mann and Hogness, 1990; Scanga *et al.*, 1993; Zhao *et al.*, 1993). These results demonstrate the role that these genes play in identity control of appendage development at the late stages. Thus, mutations in certain genes can disturb the initial state of determination even at relatively late stages of larval development and induce formation of homeotic structures. Among such mutations, locus ss^a attracts special attention. It is known that mutations at this locus result in the transformation of distal structures of antennae into the tarsal segments of mesothoracic legs, fusion of tarsal segments, and a decrease in the size of bristles (Balkaschina, 1929; Grigliatti and Suzuki, 1971; Struhl 1982). The extent of transformation of the antennal structures into tarsal structures and disturbances in tarsal segmentation depend on the temperature during development of third instar larvae (development at 18°C enhances, whereas growth at 28°C weakens the mutant phenotype (Grigliatti and Suzuki, 1971; Mglinetz, 1976). Moreover, the extent of fusion of tarsal segments depends directly on the extent of inhibition of ss^a gene expression, because the expression of the corresponding trait in hemizygotes is greatly enhanced (Bownes *et al.*, 1979; our observations). The transformation of antennal structures into tarsal structures and the fusion of tarsal segments caused by *ssa* gene mutation, demonstrate that this gene has a role in the identity control of segmentation along the body axis and in the distal-proximal axis of ventral appendages. The transformation of antennal structures into tarsus as a result of ectopic expression of *Ant, Scr,* and *Ubx* genes resembles the ss^a phenotype and has a distal-proximal orientation (Schneuwly *et al.*, 1987; Gibson and Gering, 1988; Gibson *et al.*, 1990; Mann and Hogness, 1990; Scanga *et al.*, 1993; Zhao *et al.*, 1993). This suggests that an interaction occurs between these genes and ssa during the process of appendage formation. The possibility that the *Antp* and *ssa* genes interact during the formation of homeo-tarsus instead of arista was analyzed

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in the mosaic structures formed by cells homozygous for the loss-of-function mutation in the *Antp* gene (Burgess and Dunkan, 1990). These investigations showed that *Antp* does not play a role in the formation of homeo-tarsus in ss^a flies. However, these experiments did not address the question of interactions between homeo genes and ssa during the formation of appendages in distal-proximal orientation. The change in the number of sex comb teeth in strong ss^a alleles is indirect stet that such interactions occur. This suggests that *Scr* gene increases influence on the formation of distal leg structures in males. Taking these facts into account, we designed a study to determine whether indeed ss^a interacts with genes of homeotic complexes. Our results suggest that the *ssa* gene is capable of repressing the effect of *ANT-C* and *BX-C* gene products on the formation of distal structures of antennae and leg.

Results

Isolation of ss^{aSc} *and* ss^{a40aw} *alleles*

New ss^a alleles were isolated from a temperature-sensitive line ss^{a40a} (Grigliatti and Suzuki, 1971). Apparently this line was originally heterogeneous because after many years of maintaining the stock the temperature dependence of the arista transformation has been lost, although the hemizygotes have still preserved the temperature dependence of the segmentation (Fig. 3).

Mutation ss^{aSc} was obtained by crossing between ss^{a40a} and the dysgenic strain *w oc/FM4* (Kuzin *et al.*, 1991). In addition to the highly expressed classical traits of a mutation in the ss^a locus (i.e., reduced size of bristles, transformation of distal antennal parts into the tarsal segments of the mesothoracic leg, and fusion of tarsal segments in legs and in transformed aristae), this allele is also characterized by amplification of the sex combs on the first leg in males (Fig. 1). The expressivity of these traits in homozygous ss^{aSc} flies is comparable with that in the hemizygous ss^{a40a} flies originally described (Bownes *et al.*, 1979; own unpublished data), suggesting that ss^{aSc} is a more severe loss-of-function allele. The obvious convenience of the homozygous strain, compared with the hemizygous one, prompted us to use this allele for studying the role of the gene ss^a in appendage segmentation.

The allele ss^{a40aw}, selected by us from ss^{a40a}, in homozygotes is not sensitive to temperature and shows a minimal degree of transformation of arista to tarsus (Fig. 3).

Transplantation of leg imaginal discs from Oregon R and ss^{aSc} *larvae of different ages into ready-to-pupate larvae*

It is known that the temperature sensitive period in ssa mutants is in the middle of the third instar period (Grigliatti and Suzuki, 1971; Mglinetz, 1976). In order to compare it with the time period when leg imaginal discs are competent to form different tarsus segments, we undertook transplantation experiments. First leg imaginal discs were isolated from male third instar larvae of different ages and transplanted into larvae ready to pupate. The level of hormones in such larvae does not allow the transplanted imaginal discs to continue development, and they go into differentiation prematurely, as do the recipient larvae imaginal discs.

When leg imaginal discs were dissected from *Oregon R* and ss^{aSc} larvae 0 h and 3 h after the second molt and transplanted into ready-to-pupate Oregon R larvae, no formation of imago structures was observed. However, we did see development of leg structures when the discs were dissected from both strains 7 h after the second molt. In these transplants, the development of tarsal structures was limited to the formation of the claw and bristles of the distal segment. (Development of other parts of the transplanted leg will not be discussed here).

Transplantation of discs dissected at the later stages of larval development resulted in formation of more proximal tarsal structures compared with the discs from the earlier stages. However, the structures formed in the mutant transplants looked different to those of the wild type. Transplanted imaginal discs obtained from *Oregon R* larvae 12 h after the second molt, formed one distal and one proximal tarsal segment (Fig. 2A), whereas transplanted discs of ss^{aSc} larvae of the same age formed tarsal structures without any segmentation. In both cases, proximal tarsal segments had sex combs with 8-10 teeth (Fig. 2B). When *Oregon R* discs obtained 17 h after the second molt were transplanted, they formed 4-5 tarsal segments. The first segment of the tarsus had the sex comb with 8-10 teeth. When discs from the first pair of legs of ss^{aSc} males obtained 17 h after the second molt were transplanted, they formed completely unsegmented tarsi with 10-30 sex comb teeth. First leg imaginal discs dissected from *Oregon R* larvae 27 h after the second molt formed all five tarsal segments after transplantation, with the basitarsus bearing a sex comb with 10 teeth (Fig. 2C). In a similar experiment with imaginal discs dissected from ss^{aSc} larvae, completely unsegmented tarsi were formed (Fig. 2 D). They had more then 40 sex comb teeth located much closer to the claw, as compared to the wild-type tarsi in which the claw and the sex comb are separated by four segments.

Our observations suggest that cells competent to form specific tarsal structures appear in the leg imaginal discs in specific succession. Cells competent to form distal structures appear first, followed by cells that give rise to the proximal parts of the tarsus, and finally, cells competent to produce middle segments of the tarsus appear. In ss^{aSc} imaginal discs, proliferation of cells that form the metatarsus is suppressed, whereas cells that give rise to the proximal parts of the tarsus proliferate at an excessive rate.

Tarsal segmentation in different ss^a *mutants*

The trait of incomplete segmentation has very different penetrance and expressivity in various ss^a alleles. Among the mutant alleles used in our study, the strongest phenotypic expression was observed in ss^{aSc}: tarsal segments were fused even in homozygous flies (Fig. 1), whereas in the case of ss^a, ss^{a40a}, or ss^{a40aw}, incomplete segmentation was detected only in hemizygous flies (Figs. 3,5 and data not shown for homozygous ss^a). It is remarkable that 100% of homozygous flies carrying the ss^{a40aw} allele show only very weak transformation into tarsus. However, the hemizygous phenotype is very similar to that of ss^{aSc} flies (Figs. 1 and 3). Segmentation is more strongly suppressed in hemizygous flies grown at a lower temperature (18°C), as compared with homozygous flies grown at a higher temperature (28°C).

Since segmentation is more severely disturbed in hemizygotes and at low temperatures, in comparison with the more normal segmentation that occurs at higher temperatures or in flies with an increased dosage of gene ss^a, we conclude that the extent of tarsal segmentation depends on the activity of the ss^a gene. At the same time, tarsal segments of different legs in ss^a alleles were fused to a different extent (Fig. 3 compares hemizygotes for ss^{a40a} and ss^{a40aw}). Specifically, fusion of segments was less manifested in the second legs, as compared with the first, third, and homeotic

tarsus on the antenna. This suggests that the action of ss^a on the segmentation of the tarsi is a result of interactions with some other genetic systems and that these interactions determine the final phenotype. Various genes of the *ANT-C* and *BX-C* have different expression patterns in developing imaginal discs and, probably, combinations of domains of these genes in imaginal discs determine the phenotype and the identity of the appendages in the adults. We tested whether interaction of ss^a with the *ANT-C* and *BX-C* homeotic genes determine the final phenotype of the developing leg.

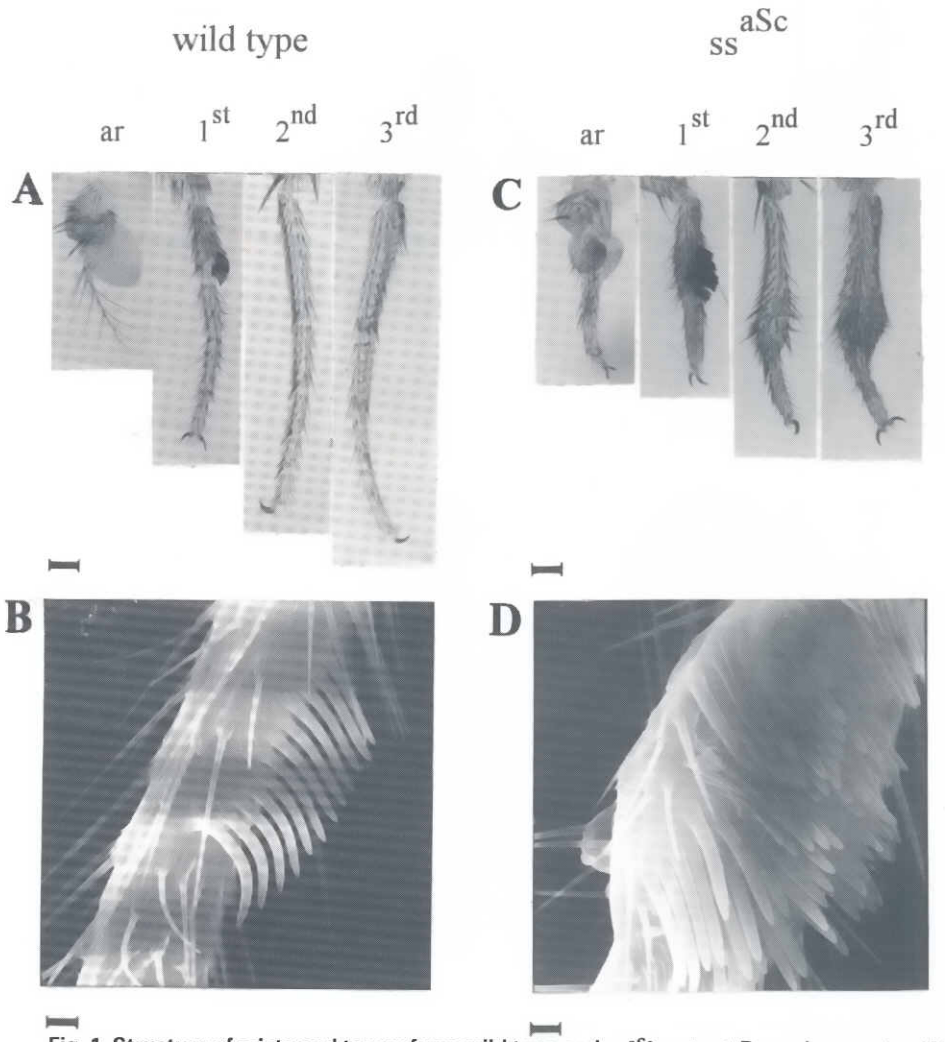

Fig. 1. Structure of arista and tarsus from wild type and ss^{aSc} mutant *D. melanogaster*. (**A**) *Structure of arista and tarsus wild type: ar- antenna with arista, 1st-tarsus of 1st leg of male fly, 2nd- tarsus of 2nd leg, 3rd- tarsus of 3rd leg.* (**B**) *Sex combs of the first tarsal segment of the first leg of the male wild type fly.* (**C**) *Structure of transformed aristae into the tarsus and fusion of tarsal segments in leg homozygous ss^{aSc} fly.* (**D**) *Amplification of sex comb of the first tarsal segment of the first leg of the male homozygous ss^{aSc} fly. Bars: (A,C), 25 μm; (B, D), 250 μm.*

Effects of disruption of* BX-C *and ANTP-C expression on tarsal segmentation in ss^a mutants

Using different alleles of ss^a, we investigated the possible involvement of the products of homeotic genes of the *ANTP-C* and *BX-C* in the segmentation of tarsi.

Comparison of the ss^{a40aw} hemizygous flies in different *ANT-C* and *BX-C* gene backgrounds has demonstrated that following development at 28°C the changes in the segmentation of the tarsal segments of the third leg were more profound in $ss^{a40aw}/Df(3r)sbd104$ flies with a normal *ANT-C* and *BX-C* background than in $ss^{a40aw}/Df(3R)P10$ flies carrying only one dose of the *Ubx* gene (Fig. 3). (Segmentation in flies that developed at 18° was disrupted completely, hence no differences are seen.) Flies with $ss^{a40aw}/TM6,ss^a$ Ubx genotype displayed a similar improvement of segmentation in the tarsi of the third leg.

To analyze the effect caused by ectopic expression of *Ubx* in the second thoracic segment, we compared tarsal segmentation in hemizygous flies $ss^a/Df(3R)sbd^{104}$ and $ss^a\ bx^1\ Cbx^1/Df(3R)sbd^{104}$. Mutation *Cbx* causes ectopic expression of *Ubx* in the second thoracic segment (Cabrera *et.al.*, 1985; White and Akam, 1985).

Flies *ssa* $bx^1\ Cbx^1/Df(3R)\ sbd104$ are characterized not only by the usual reduction of the posterior part of the wing, caused by gain-of-function mutation Cbx^1, but also by deterioration of tarsus segmentation of the second leg (Fig. 4). (Apparently this deterioration may be caused by ectopic expression of *Ubx* in second thoracic segment).

Since $ss^a\ bx^3\ bxd^{51j}/Df(3R)P10$ do not emerge as imagoes, we analyzed their phenotype by dissecting late pupae. As a result of loss-of-function mutations bx^3, bxd^{51j}, they have four pairs of legs. (*bx* and *bxd* mutations usually lead to the absence of or a large decrease in *Ubx* expression in the third thoracic and first abdominal segments (Gonzalez-Reyes *et al.*, 1990; González-Reyes and Morata, 1990). Thus, formation of the homeo leg is most probably due to the ectopic expression of the *Antp* gene in the abdominal segments caused by the decreased expression of the *Ubx* gene. Segmentation was most severely disturbed in the fourth pair of legs, and at the same time we noticed some improvement of segmentation in the third pair of legs (Fig. 4). Such an expansion of the first tarsal segment of the homeotic leg might be caused by the cumulative effect of *Antp* and *abd-A* genes.

The comparison of the influence of loss- and gain-of function mutations in the *Ubx* gene on tarsus formation under conditions of shortage of ss^a gene products shows increased fusion of tarsal segments in gain-of-function mutations and a less severe phenotype in loss-of-function mutations.

Alteration in the expression levels of selector genes of the *ANT-C* and *BX-C* can be caused not only by mutations in these genes,

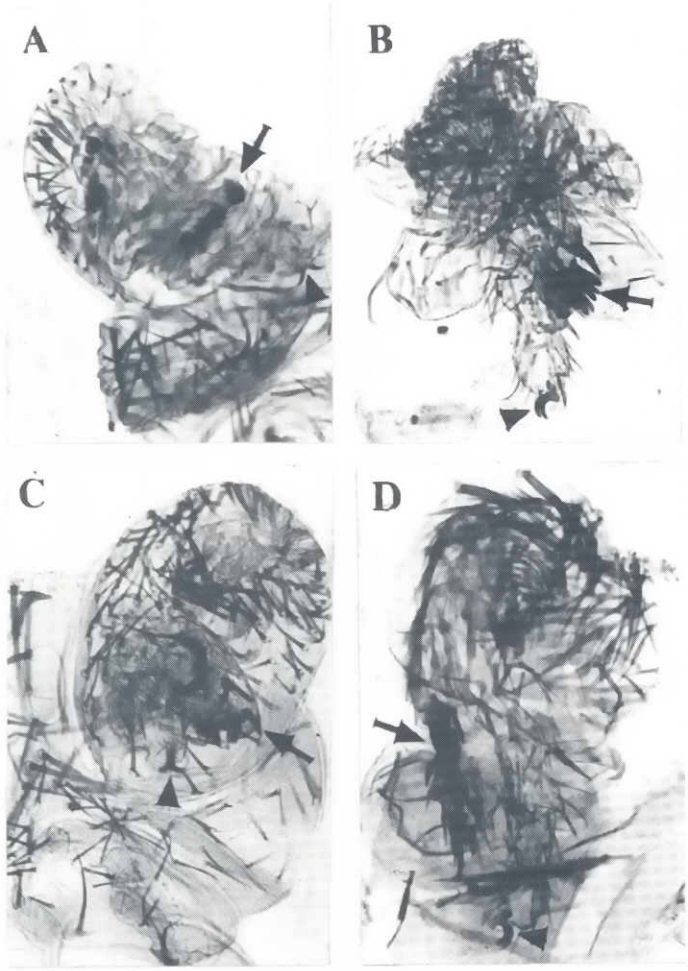

Fig. 2. Structures formed by imaginal discs of the first pair of legs that were isolated from Oregon R and ss^{aSc} larvae 12 and 27 h after the second molt and transplanted into ready-to-pupate Oregon R larvae. (**A**) *Structures formed by an Oregon R disc and* (**B**) *by an* ss^{aSc} *disc transplanted 12 h after the molt.* (**C**) *Structures formed by an Oregon R and* (**D**) ss^{aSc} *disc transplanted 27 h after the molt. The arrows indicate sex combs, the arrowheads indicate tarsal claws on the distal segments. Bar, 50 μm.*

but also by the mutations in repressor or activator genes. To further elucidate the interactions between ss^a and homeotic genes, we used fly lines with mutations of *Policomb (Pc)* and *thritorax (trx)* genes (negative and positive regulators of *ANT-C* and *BX-C* genes accordingly) (Ingham and Whittle, 1980; Struhl, 1983). Mutation Pc^3 in the background of normal ssa causes the appearance of 1-2 sex comb teeth on one of the legs of the second and third pair.

Flies $ss^{a40aw}/Df(3R)P10,st,in,ri,Pc^3,p^p$ had sex combs on the tarsi of each leg in males. At lower temperatures, the number of comb teeth increased up to several dozens, and segmentation of all legs was highly suppressed (Fig. 3). Since *Pc* is a negative regulator of all homeotic genes, the observed disturbances of segmentation were apparently caused by the cumulative effects of increased expression of *Antp, Scr* and *Ubx* genes under the conditions of decreased amount of the ss^a gene product. This conclusion is supported further by the results of overexpression of the *Scr* gene, which causes the appearance of an extraordinarily large number of sex comb teeth on the segments of the second and third thoracic legs (Fig. 3).

When a mutation in trx was introduced in compound with the *Pc* gene in flies $w;trx^{B22},ss^{a40aw}/Df(3R)P10,st,in,ri,Pc^3,p^p$, negative effects of *Pc* on the formation of tarsal structures were completely neutralized (data not shown).

Taking into account the results of disturbances in tarsal development, caused by ss^a mutations in the background of mutations, which alter the expression of *ANT-C* and *BX-C* complexes, we conclude that one of the functions of the ss^a gene is the suppression of the homeotic gene influence on the development of distal tarsal structures.

Effect of overexpression of ANTP-C genes on tarsal segmentation in ss^a *mutants*

To understand the repressing effect of ss^a on *ANTP-C* genes, we analyzed the effect of overexpression of *Antennapedia* (*Antp*), *Deformed* (*Dfd*), *labial* (*lab*), and *proboscipedia* (*pb*) *genes* in the background ss^a mutants.

Larvae of the flies carrying *ANTP-C* genes under the control of the heat-shock promoter were transferred to 37°C at different stages of development. An analysis of the tarsal structures in flies w; $P(W+ hsp70::Antp);ss^{aSc}/TM6, ss^{a-}$ showed that the induction of hs*Antp* during 7-17 h of the third instar results in severe tarsal defects; in extreme cases, tarsal structures resembled mini-tibia (Fig. 5). Moreover, antennal structures looked unusual due to overgrowth of the second and third antennal segments and proximal structures of the homeo tarsus .

Induction of *hsp Antp* in the background of normal ss^{a+} (as described by Gibson and Gering, 1988; our observations) does not influence formation of different tarsal structures , but often causes thickening of the arista and enlargement of the second and third antennae segments.

Induction of *hsDfd* was performed with $P[hsDfd,ry^+]2;ss^{aSc}/TM6,ss^{a-}$ larvae 7-17 h after the second molt. The resulting flies showed an $P[hsDfd,ry^+]2;ss^{aSc}/TM6,ss^{a-}$ overgrowth of the third antennal segment and of proximal parts of the homeo tarsus (Fig. 5). In legs, we also observed enlargement of the proximal part of the tarsus. In control experiments, in which *hsDfd* was expressed against the normal ss^a background, no abnormalities of antennal and leg structures were detected (McGinnis *et al.*, 1990; our observations).

We analyzed the phenotypes of different ss^a mutants, which also carried *lab* and *pb* under a heat-shock promoter, after heat shock was applied 7-17 h after the second molt. Induction of hs*lab* in hemizygous ss^{aSc} mutants led to shortening of tarsi in all antennal and leg structures (Fig. 5) to such an extent that antennal tarsi were almost lost. On the other hand, the third segment of the antennae had structures with multiple similar bristles, which were similar to occipital bristles.

Induction of hs*lab* in hemizygous ss^{a40aw} also caused an overgrowth of the third antennal segment, but without the formation of multiple bristles. We did not observe any significant effects of hs*lab* expression on tarsal segmentation in these flies. The induction of *hslab* in the background of the ss^{a+} gene, did not cause any changes in the tarsal leg structures, but only led to some thickening of the arista.

Induction of hs*pb* in hemizygous ss^{a40aw} larvae led to shortening of the distal parts of the tarsus. Clearly manifested in antennae, this

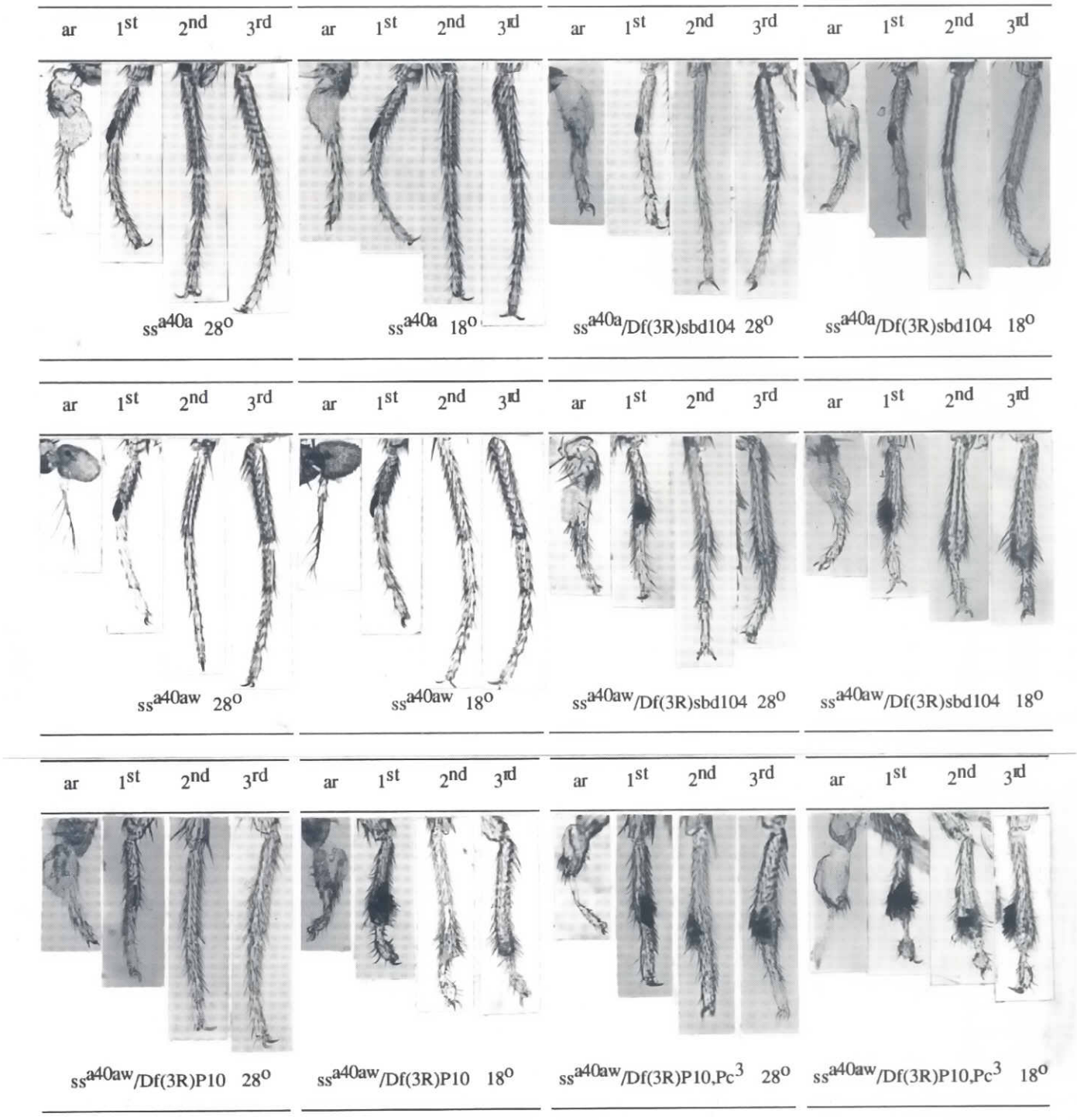

Fig. 3. Tarsal structures formed on antennae and legs in hemi- and homozygotes for mutant *ss^{a40a}* and *ss^{a40aw}* alleles that developed at different temperatures. *(ar) antenna with arista; (1st), (2nd), and (3rd), legs of corresponding pairs; Df(3R)sbd104, deletion affecting locus ss^{a}. Df(3R)P10, deletion affecting ss^{a} and Ubx genes. Bar, 25 μm.*

effect was less pronounced in legs of the first and second pairs and was virtually absent in legs of the third pair (Fig. 5). In antennae, we observed almost complete loss of tarsal structures, the only remaining part being claws growing from the third antennal segment. The induction of *hspb* in the background of normal *ss^{a+}* did not cause any changes in leg development. However, in such flies, we observed some thickening of the arista, which slightly increased after temperature shock.

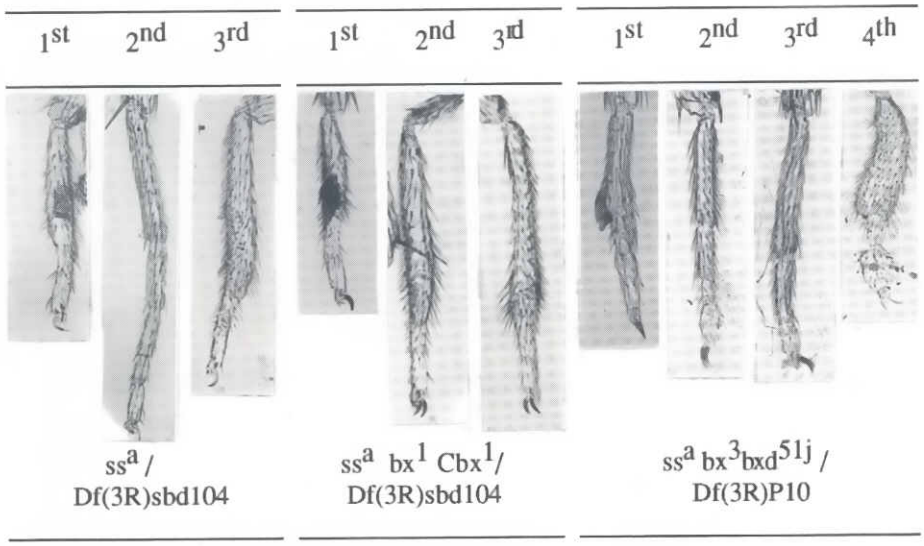

Fig. 4. Tarsal structures formed on antennae and legs in hemizygotes for *ssa* and *ssa40aw* in the background of different mutations affecting the pattern of expression of *ANT-C* and *BX-C* genes. *(ar) antenna with arista; (1st), (2nd), and (3rd), legs of corresponding pairs; Df(3R)sbd104, deletion affecting locus ss^a. Df(3R)P10, deletion affecting ss^a and Ubx. Bar, 25 μm.*

The comparison of the consequences of the *hsAntp, hsDfd, hslab, hspb*, gene induction in the background of normal ss^{a+} and its mutant alleles ss^{a40aw} and ss^{aSc} leads to the conclusion that the ss^a gene restrains the influence of homeo-genes on the tarsal structure development. Since transcription of homeotic genes was controlled by the hsp70 promoter in these experiments, we conclude that the ss^a gene products repress homeotic genes at the post-transcription level.

Discussion

The results obtained during transplantations of the leg imaginal discs suggest that the deficiency in the ss^a gene product, which results from the mutation ss^{aSc}, leads to excessive proliferation of cells that become competent to form proximal segments of the tarsus. This is accompanied by the determination of a group of cells to form segments located between the distal and proximal tarsal segments. This conclusion is based on the fact that in the beginning of third instar imaginal discs ss^{a+} and ss^{aSc} flies have equal abilities to form tarsal structures. By 27 h after the molt, however, ss^{a+} discs are capable of forming all tarsal segments, whereas ss^{aSc} discs can produce a nonsegmented tarsus containing certain structures of the distal segment and multiplied structures of the proximal segment. Cells competent to form intermediate structures do not appear in ss^{aSc} discs. Differences between normal and mutant larvae in the establishment of competence to form tarsal structures in cells of leg imaginal discs coincide with the temperature-sensitive period of ss^a mutations (Grigliatti and Suzuki, 1971; Mglinetz, 1976) Moreover, the period of autonomous expression of these mutations terminates at the same time (Ginter *et. al.*, 1974; Postlethwait and Girton, 1974).

The phenotype depends on the level of expression of the ss^a gene since the severity of segmentation disturbances increases during conversion of ss^a alleles from the homozygous to the hemizygous state and during the shift from high to low temperature of development.

These observations do not explain the mechanisms of ss^a involvement in appendage development, but they do confirm the important role played by this gene during this period.

At the same time, the analysis of tarsal structures in flies carrying different mutant ss^a alleles expressed in the background of different *ANT-C* and *BX-C* genes showed that amplification of the proximal tarsal structures depends not only on the level of ss^a expression, but also on the pattern and level of expression of *ANT-C* and *BX-C* genes. This is demonstrated by the analysis of the tarsal structures formed in different mutants, in which the expression of *ANT-C* and *BX-C* genes in the ss^a background is disturbed. In ss^{aSc}/ss^{aSc}, $ss^{a40aw}/Df(3R)sbd^{104}$ flies with a normal *ANT-C* and *BX-C* pattern, disturbances of tarsal segmentation in the first and third pairs of legs are more serious than in the second pair (Fig. 1 and 3). It is known that the *Antp* gene is expressed in imaginal discs of all three pairs of legs (Levine *et al.*, 1983; Stroeher *et al.*, 1986; Wirz *et al.*, 1986). In addition to *Antp*, genes *Scr* and *Ubx* are expressed in discs of the first and third pairs of legs, respectively (White and Wilcox, 1985; Brower, 1987; Martinez-Arias *et al.*, 1987; Botas *et al.*, 1988; Glickman and Brower, 1988; Pattatucci and Kaufman, 1991) We believe an increased contribution of proximal tarsal structures in the first and third pairs of legs can be explained by the cumulative effects of genes *Antp* and *Scr* in the first case and *Antp* and *Ubx* genes in the second. A decreased Ubx dosage in $ss^{a40aw}/Df(3R)P10$ flies, which lack the whole Ubx domain and part of the abd-A domain, and $ss^{a40aw}/TM6,ssa^-,Ubx$ flies leads to milder disturbances of tarsal segmentation in the third pair of legs, compared to those in the second pair. Greater severity of such disturbances in the second pair of legs in $ss^a\ bx^1\ Cbx^1/Df(3R)sbd^{104}$ flies (Fig. 4) can be explained by the ectopic *Ubx* expression in imaginal discs of the second thoracic segment, which results from the *Cbx* mutation (Cabrera *et.al.*, 1985; White and Akam, 1985)

We observe a significant suppression of tarsal segmentation in the fourth pair of legs of $ss^a\ bx^3\ bxd^{51j}/Df(3R)P10$ flies (Fig. 4), (bx and bxd mutations usually lead to the absence of or a large decrease in Ubx expression in the third thoracic and first abdominal segments (Gonzalez-Reyes *et al.*, 1990; González-Reyes and Morata, 1990). Thus, formation of the homeo-leg is most probably due to the ectopic expression of the *Antp* gene in the abdominal segments caused by the decreased expression of the *Ubx* gene

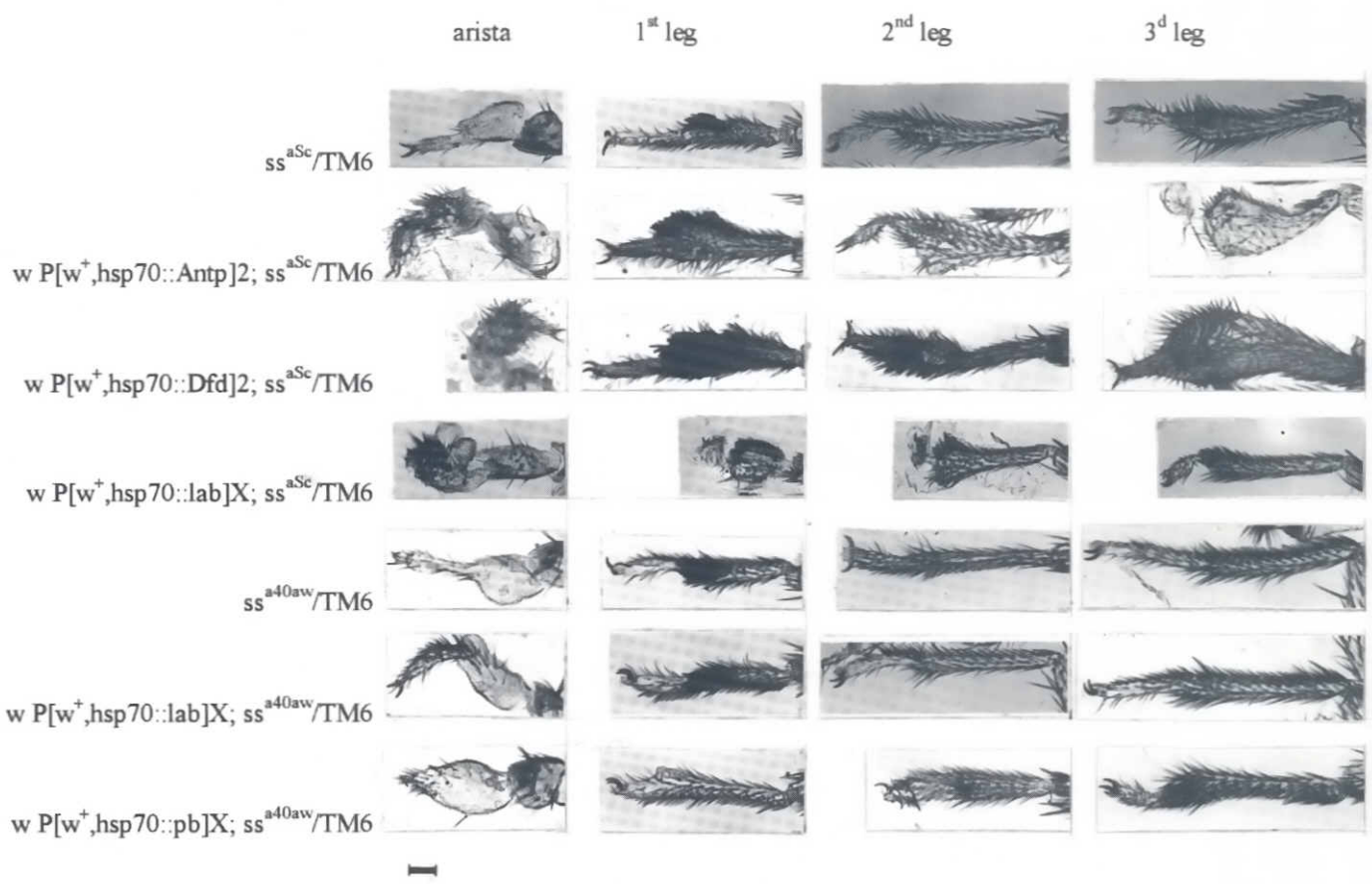

Fig. 5. Tarsal structures formed on antennae and legs in hemizygotes for ssaSc and ssa40aw in the background of different ANT-C genes after ectopic induction under the control of the heat shock promoter. *(ar) - antenna with arista; (1st), (2nd), and (3r) - tarsi of the first, second and third pairs of legs. Bar, 25 μm.*

(Hafen *et al.*, 1984; Carrol *et.al.*, 1986). We attribute the significant suppression of tarsal segmentation in the fourth pair of legs to a cumulative effect of *Antp* and *abd-A genes*. Apparently, a «proximal» effect of *abd-A* is stronger than that of *Ubx*.

An extreme case of suppressed tarsal segmentation is observed in *ss^{a40aw}/Df(3R)P10,st,in.ri,Pc^3,p^p* flies (Fig. 3). An increased «proximalization» of tarsal structures in *ss^{a40aw}/Df(3R)P10,st,in.ri, Pc^3,p^p* flies, as compared with *ss^{a40aw}/Df(3R)P10* flies, is caused by a mutation in the *Pc* gene, a negative trans-regulator of *ANT-C* and *BX-C*. Mutation *Pc^3* causes the overexpression of *Scr* and *Ubx* in all three pairs of leg imaginal discs (Busturia and Morata, 1988; Pattatucci and Kaufman, 1991). In the presence of a normal allele *ss^a*, expression of these genes in legs is minimal, that is, one or two sex comb teeth appear on one or two meso- or metathoracic legs in 75% of flies. In *ss^{a40aw}/Df(3R)P10,st,in,ri,Pc^3,p^p* flies, in addition to most severe disturbances of tarsal segmentation, 100% of thoracic tarsi have an excessive number of sex comb teeth. This suggests that one of the functions of gene *ss^a* is probably to restrict influence of gene *Scr* in distal regions of the appendages. At the same time, the total sum of data obtained in this study suggests that this function of *ss^a* is universal and that it concerns all genes of *ANT-C* as well as the *Ubx* gene.

Ectopic expression in third instar larvae of genes *hsAntp*, *hsDfd*, *hslab*, and *hspb* in the background of wild type *ss^a* results only in insignificant thickening of aristae and does not affect the formation of tarsal structures. However, when the function of *ss^a* is repressed, the expression of these genes results in an overgrowth (for *Antp* and *Dfd*) or supression of growth (for *lab* and *pb*) of proximal tarsal structures and of third and fourth antennal segments, reflecting a sharp increase in a «proximal» effect on the development of distal structures. These results show that *ss^a* performs an important function in the network of genes controlling the proximal-distal pattern of leg structures formed in imagoes. This function is aimed at restricting the effects of products of *ANT-C* and, probably, *BX-C* gene in cells that acquire the competence to form the distal structures of ventral appendages. The repressive function of *ss^a* is probably unrelated to the control of transcription of *ANT-C* genes, because it is fully realized with respect to the heat-shock inducible genes. We suggest that the mechanism of this repression involves competition of *ss^a* product with *ANT-C* products on the target genes, whose expression is responsible for the formation of a particular pattern of distal limb structures. This occurs during the third instar larvae, when cells of leg and antennal imaginal discs acquire the competence to form these structures, and coincides with the period of sensitivity of gene *ss^a* to temperature (Grigliatti and Suzuki, 1971; Mglinetz, 1976). However, these events are preceded by a period in a 2nd instar larvae when ectopic induction of genes *Antp*, *Scr* and *Ubx* can result in transformation of arista

into tarsus (Schneuwly *et al.*, 1987; Gibson and Gering, 1988; Gibson *et al.*, 1990; Mann and Hogness, 1990; Scanga *et al.*, 1993; Zhao *et al.*, 1993). This indicates that the expression of gene ss^a in cells of the leg and antennal imaginal discs at the end of the 2nd instar depends on the presence of *ANT-C* genes products. Apparently, ss^a might be an indirect target gene for *ANT-C* and *BX-C* genes during this period, and the pattern of their expression predetermines that of ss^a expression during subsequent development of imaginal discs. As a result, the pattern of ss^a expression in leg discs, where genes *Scr*, *Antp*, and *Ubx* are expressed, becomes favorable for the formation of tarsal structures. At the same time, in antennal imaginal discs with predominant expression of genes *Dfd* and *lab*, ss^a is expressed in a way that promotes the formation of aristae. At later stages, the products of *ANT-C* and *BX-C* genes apparently cannot affect ss^a expression, because the absence of genes *Antp*, *Scr*, and *Ubx* genes has no effect on the formation of tarsus (Struhl, 1981,1982; Abbott and Kaufman, 1986; Burgess and Duncan, 1990). Taken together, the data on the expression of homeotic genes and their regulators in the background of a normal and mutant ss^a gene demonstrate that products of the ss^a gene are capable of repressing the effect of *ANT-C* and *BX-C* gene products on downstream genes involved in the formation of distal structures of antennae and legs.

Materials and Methods

Transplantations of imaginal discs

The basis of the method used in these experiments was described by Ephrussi and Beadle (1936). Leg imaginal discs were removed from third instar larvae 0, 3, 7, 12, 17, and 27 h after the second molting and transplanted into ready-to-pupate larvae. After metamorphosis of host larvae, differentiated implants were removed from their abdomens, placed on a glass slide into a drop of gum arabic solution, and covered with a coverslip for microscopic examination (Lillie, 1965).

Microscopic analysis of tarsal structures

To examine tarsal segmentation, adult flies were boiled for 10 min in 10% KOH solution, washed in water, and transferred into 50% glycerol for one day. Flies were then embedded in 100% glycerol under a coverslip, and preparations were examined using a AHBT-3 microscope (Olympus) at magnification x330.

Drosophila lines and crosses used in the study

Depending on the specific purposes of the experiment, flies were raised on a standard medium at 18°, 23°, 25° or 28°C. The following ssa alleles were used in this study: ss^a, ss^{a40a}, ss^{a40aw}, ss^{aSc}. Hemizygotes for different ss^a alleles in the background of normal genes *ANT-C* and BX-C were obtained in crosses with stet $Df(3R)sbd^{104}/T(2;3)ap^{Xa}$ received from the Bloomington collection. Tarsal structures were studied in flies $Df(3R)sbd104/ss^a$.

Hemizygotes for ss^{a40aw} with half a dose of the *Ubx gene* were obtained in crosses with flies of the line *Df(3R)P10/TM1* received from the Bloomington collection. Tarsal structures were studied in flies $Df(3R)P10/ss^{a40aw}$. Hemizygotes for ss^{a40aw} expressed in the mutant background of the *Pc gene* were obtained in crosses with flies $Df(3R)P10, st\ in\ ri\ Pc^3\ p^o / TM6B$ received from the Bloomington collection. For investigation of tarsal structures, the flies $Df(3R)P10,st\ in\ ri\ Pc^3\ pp/ss^{a40aw}$ were used .

Hemizygotes for ss^{a40aw} expressed in the background of *trx* gene were obtained using the allele *trxB22* induced by P-element mutagenesis received from J.Keninson. For this purpose, $w;\ trx^{B22}/TM6C$ flies were crossed with $w;\ ss^{a40aw}$ flies. Then $w,\ trx^{B22}/ss^{a40aw}$ females selected in F_1 were crossed with $w,\ Sb/TM6,ss^{a-}$ males. In F_2, crossover males and females $w;\ trx^{B22}\ ss^{a40aw}/TM6,ss^{a-}$ were selected for analysis.

Hemizygotes for ss^a in the *bx, bxd* background were obtained in crosses between $ss^a\ bx^3\ bxd^{51j}/TM1$ and $Df(3R)P10/TM1$ flies (both lines received from the Bloomington collection). The flies of the genotype $Df(3R)P10/ss^a\ bx^3\ bxd^{51j}$ were analyzed.

Hemizygotes for ss^a in the *Cbx* background were obtained in crosses between $ss^a\ bx^1\ Cbx^1/T(2;3)ap^{Xa}$ and $Df(3R)sbd^{104}/T(2;3)ap^{Xa}$ flies (both lines received from the Bloomington collection), and the flies $Df(3R)sbd104/ss^a\ bx^1\ Cbx^1$ were analyzed.

To study *hs Antp* expression in the ss^a background, we used a transformed line $yw^{67cz};P[w^+,hsp70::Antp]$ kindly supplied by Dr. M. Scott. Males of this line were crossed with $w;Sp/CyO;ss^{aSc}$ females. Crosses were then made between $w/yw^{67cz};P[w^+,hsp70::Antp]/CyO;ss^{a+}/ss^{aSc}$ flies selected in F_1 and between $w;P[w^+,hsp70::Antp];ss^{aSc}$ flies selected in F_2. During the period from 7 to 17 h after the second molt, F_3 larvae were placed for 40 min in a water thermostat at to 37°C. In this experiment flies of the initial lines developing under identical conditions were used as controls.

For the expression of *hspb* in the ss^a background, we used hspb line $P[w+,hsp70::pb^3]X$ which was kindly provided by Kaufmann and corresponds to the poor expressing line $P[w^+,hsp70::pb^3]X$ with insertion into the X-chromosome (Cribbs *et. al.*, 1995). Females of this line were crossed with $C(1)M3,y^2\ bb/FM7;Sb/TM6,ss^{a-}$ males, and $P[w^+,hsp70::pb^3]x/FM7;3+/TM6,ss^{a-}$ females selected in F_1 were crossed with ss^{a40aw} males. During the period from 7 to 20 h after the second molt, F_2 larvae were placed for 40 min at 37°C. The effect of *hspb* expression on tarsal segmentation was analyzed by comparing F_2 males $P[w^+,hsp70::pb^3]X;ss^{a40aw}/TM6,ss^{a-}$ with orange eyes, $X+;ss^{a40aw}/TM6,ss^{a-}$ with red eyes, and $P[w^+,hsp70::pb^3]X;ss^{a40aw}/3+$ with orange eyes as a control.

For analyzing *hslab* expression in ss^a background, we used a transformed line $P[w^+,hsp70::lab]X$ kindly supplied by Dr.T. Kaufmann. Females of this line were crossed with $C(1)M3,y^2\ bb/FM7;Sb/TM6,ss^{a-}$ males, and females $P[w^+,hsp70::lab]X/FM7;3+/TM6ss^{a-}$ selected in F_1 were crossed with ss^{aSc}, ss^{a40aw} males. During the period from 7 to 20 h after the second molt, F_2 larvae were placed for 1 h at 37°C. The effect of *hslab* expression on tarsal segmentation was studied by comparing the results of crosses of F_2 males $w;P[w^+,hsp70::lab]X;ss^{aSc}/TM6,ss^{a-}$ (orange eyes), $X+;ss^{aSc}/TM6,ss^{a-}$ (red eyes), and $w;\ P[w^+,hsp70::lab]X;ssaSc/3+$ (orange eyes) with ss^{aSc} females and of the same males with ss^{a40aw} females.

The expression of *hsDfd* against the ss^a background was studied using a transformed line $P[hsDfd,ry^+]2;ry^{506}$ received from the Bloomington collection. Flies of this line were crossed with $Sp/CyO;ss^{aSc}$ flies, and $P[hsDfd,ry^+]2/CyO;ry^{506}/ss^{aSc}$ flies were selected in F_1. After crossing F_1 flies, $P[hsDfd,ry^+];ss^{aSc}$ flies were selected in F_2. Larvae of the line obtained in this way were placed at 37°C for 30 min (7-20 h after the second molt) in order to induce *hsDfd*. The effect of *hsDfd* expression on tarsal segmentation was studied in comparison with $P[hsDfd,ry^+]2;ry^{506}$ and ss^{aSc} flies developing under identical conditions.

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