

A NOTE ON SOME ALLELES OF ARISTAPEDIA

By C. H. WADDINGTON AND R. CLAYTON

Department of Animal Genetics, University of Edinburgh

(With Four Text-figures)

INTRODUCTION

It often seems to be the case that the members of a series of multiple alleles differ only in the efficiency with which they carry out some developmental process, and it may then seem plausible to suggest that the differences between the actual genes are themselves of a quantitative nature (Stern, 1930; Muller, 1932). If this were so, we should expect to find that where a set of alleles affects a number of different characters of the adult, the seriation of the genes with respect to any one character was parallel to that with respect to any other. Moreover, when it is possible to alter the grade of expression ('expressivity') of the alleles by environmental means, it should be possible to cause the expression of one of the weaker alleles, in some environment, to resemble exactly that of a stronger allele in some other environment, or vice versa.

Several cases are already known in which such simple relations do not hold. Nevertheless it seems worth while to record the following observations on some alleles of the well-known aristapedia locus of *Drosophila melanogaster*, since they not only provide a clear example of non-quantitative differences between alleles, but offer a number of other points of interest, which will be referred to in the discussion. The most commonly recognized effect of these genes is to cause the arista, or even the antenna as a whole, to develop into a leg-like organ. Several alleles are known, some having a stronger and others a weaker effect. Of the commoner ones, aristapedia-Bridges (ss^{aB}) is usually considered a 'weak' allele, since at the temperatures at which most laboratory stocks are kept, its effect on the arista is to produce only a slight thickening of the base, whereas under the same conditions, the original allele (ss^a) or still more such an allele as aristapedia-Spencer (ss^{aSp}) causes the development of a large antennal tarsus. At the same time, the effect of the alleles is by no means confined to the antenna. Many of them can be seen, in normal cultures, to have some effect on the tarsi, which tend to be reduced in length and to exhibit only an incomplete and often irregular segmentation. In some alleles there is also a slight reduction in length of the macrochaetae; and they act as alleles of spineless (ss), the macrochaetae of the compound ss^a/ss being considerably reduced. Some alleles show still other effects. Le Calvez (1948*a, b*) has described an 'aristapedia-dominant' (ss^{aR}) associated with a chromosome aberration, which not only transforms the arista into a tarsus, but causes a hypertrophy of the basal joint of the antenna, a reduction of the ocelli, a deformation of the head, a reduction of the cephalic macrochaetae and an alteration in the attitude of the wings; it appears, however, to have no effect on the size or segmentation of the legs.

Several authors (Villee, 1943; Vogt, 1946*a*; Le Calvez, 1948*a, b*) have shown that the expression of some of the aristapedia alleles can be considerably modified by controlled temperature treatments during the late larval period. Villee showed that cold treatments (at 14.4° C.) increased the tendency for a leg to be formed in place of an arista by flies

homozygous for ss^{aB} , while heat treatments had the opposite effect both with this allele and with ss^a . Vilee claimed that the cold treatments which increased the degree of abnormality of the legs of ss^{aB} flies had no effect on those of ss^a . Vogt and Le Calvez only studied one or two other alleles, and did not investigate the effects on both aristae and legs simultaneously.

The available data are therefore somewhat scanty, but would seem to suggest that only certain developmental effects of some alleles are affected by temperature, while other effects are insensitive. This would seem a peculiar state of affairs, and some re-examination of the matter seemed called for.

EXPERIMENTS

In the experiments to be reported here, use was made of the alleles ss^a , ss^{aB} , ss^{a40a} and ss^{aSnB} . The two former originated from the stocks kept at Cold Spring Harbor, while the two latter were kindly given us by Dr Buzzatti Traverso. They had their origin in a stock of flies caught in the wild. An aristapedia allele appeared in an inbred line from this stock. It was named ss^{a40a} , and at first it showed a strong expression of the character both in the antennae and legs. After some generations, the leg effect had disappeared. On the supposition that this might be due to a gradual modification of the genetic background, an attempt was made to re-isolate the original allele by inbreeding other lines derived from the same original wild stock. This was successful, and an aristapedia allele appeared in inbred flies from a line in which *singed* was also present. This second stock was named ss^{aSnB} (Buzzatti Traverso, 1947). One might presume, however, that the aristapedia alleles present in the two stocks ss^{a40a} and ss^{aSnB} would actually be identical, the difference lying only in the genetic background. Their behaviour in the following experiments supports this hypothesis.

Eggs were collected over 3 hr. periods, and cultured at 27° C. for a varying period, after which they were given a cold treatment, which consisted in transferring them to 18° C. for 3 days; they were then returned to 27° C. to complete their development. The treatments were given at 12 hr. (treatment I), 24 hr. (II), 36 hr. (III), 48 hr. (IV) or 60 hr. (V) after laying.

The results were recorded by the use of an arbitrary system of grades. For the leg-grades, the animals were classified into five groups according to whether the tarsi were equivalent in length to 5, 4, 3, 2 or 1 normal tarsal segments. As a rule the abnormal legs were segmented into a number of joints roughly corresponding to their length, but the segmentation was usually somewhat disordered, and the legs might be swollen, with disarranged chaetotaxy, particularly in the most abnormal grades 1 and 2. On each fly, the right foreleg and right hindleg were observed, and the grades given are the means of these two observations. The aristae were classed into the eight grades described and figured by Vilee (1943), with the addition of a grade 9 for cases in which an apparently perfect foot was developed, with claws, pulvillus, etc.; antennae in which the arisal tarsus was strongly abnormal, in a manner parallel to the abnormality of the legs (e.g. in Fig. 4), were also included in grade 9.

The results of one series of experiments are given in Table 1. Some typical legs and arisal-legs are drawn in Figs. 1-4. No attempt was made to make the stocks containing the different alleles isogenic with one another, but similar experiments were repeated with a number of different stocks of each allele, and in all cases the various stocks of any one

allele behaved similarly. There is little doubt, therefore, that the differences in behaviour are due to the aristapedia alleles themselves, only slightly affected by the genotypic backgrounds.

It is clear that both the arisal and the tarsal effects are affected by temperature treatments in all alleles. It appears also that their temperature-sensitive periods are at

Table 1. Mean antennal and tarsal grades, with standard errors, of flies following cold treatment at 12, 24, 36, 48 and 60 hr. after laying

	No.	Aristal grade	Tarsal grade	No.	Aristal grade	Tarsal grade
		Female			Male	
<i>ss^{aB}</i> :						
12 hr.	10	2.1 ± 0.18	5.0	8	2.0	5.0
24 hr.	10	2.5 ± 0.22	5.0	7	1.9 ± 0.14	5.0
36 hr.	13	2.2 ± 0.12	4.8 ± 0.03	7	2.1 ± 0.14	5.0
48 hr.	8	4.6 ± 0.26	4.6 ± 0.25	8	2.9 ± 0.13	5.0
60 hr.	12	7.3 ± 0.19	2.6 ± 0.14	11	6.3 ± 0.24	3.4 ± 0.23
<i>ss^{a40a}</i> :						
12 hr.	12	7.7 ± 0.22	4.6 ± 0.14	12	7.8 ± 0.22	4.5 ± 0.57
36 hr.	3	7.7 ± 0.33	3.8 ± 0.44	8	7.4 ± 0.26	4.6 ± 0.15
48 hr.	11	8.0 ± 0.14	3.4 ± 0.27	11	7.9 ± 0.09	3.5 ± 0.32
60 hr.	10	8.8 ± 0.13	1.8 ± 0.13	10	8.2 ± 0.20	1.8 ± 0.21
		Female and male				
<i>ss^{a5mB}</i> :						
Continuous at 18° C.	9	9.0	1.1 ± 0.6			
Continuous at 25° C.	16	8.1 ± 0.09	2.69 ± 0.11			
		Female and male				
<i>ss^a</i> :						
12 hr.	20	7.8 ± 0.10	5.0 ± 0.025			
24 hr.	16	7.8 ± 0.12	5.0			
36 hr.	10	8.0 ± 0.075	5.0			
48 hr.	20	8.0	4.8 ± 0.09			
60 hr.	24	8.1 ± 0.52	4.6 ± 0.09			
Continuous at 18° C.	10	8.1 ± 0.15	4.5 ± 0.15			
Continuous at 25° C.	7	6.5 ± 0.27	5.0			

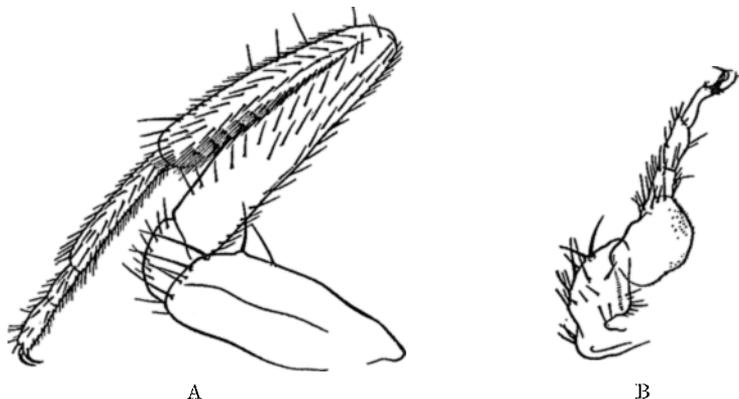


Fig. 1A, B. Foreleg and antenna of *ss^{aB}*, treatment 5 (tarsal grade 3, arisal grade 6).

least roughly the same, very little effect being produced earlier than 48 hr. after laying, although there may be a slight effect on the legs in *ss^{a40a}*. The magnitude of the effect is, however, certainly different in the different alleles. For instance, *ss^a* is much less sensitive than *ss^{aB}* both in its action on the legs and on the arista; its leg effect is also less sensitive than that of *ss^{a40a}*, and the same may be true of its arisal effect, but since this is in any

case near the maximum possible the difference is not very striking and the grading of the flies may not be very reliable.

It is important to note that the pattern of effect is different in the different alleles. Thus ss^a , in all conditions, produces a strong effect on the arista and only a weak effect on the legs; ss^{aB} , when expressed strongly enough to have an important effect on the arista, also has a considerable effect on the legs; while ss^{a40a} is, in its weaker manifestations, rather like ss^a , but when strongly expressed involves a much greater reduction in the legs. The stock ss^{aSnB} appears to be similar in its pattern of expression to ss^{a40a} , but its manifestation under the same conditions is always more extreme. It seems probable that this difference is due to the different genetic backgrounds in the two strains, but the other differences cannot easily be explained in this way, as has been pointed out above.

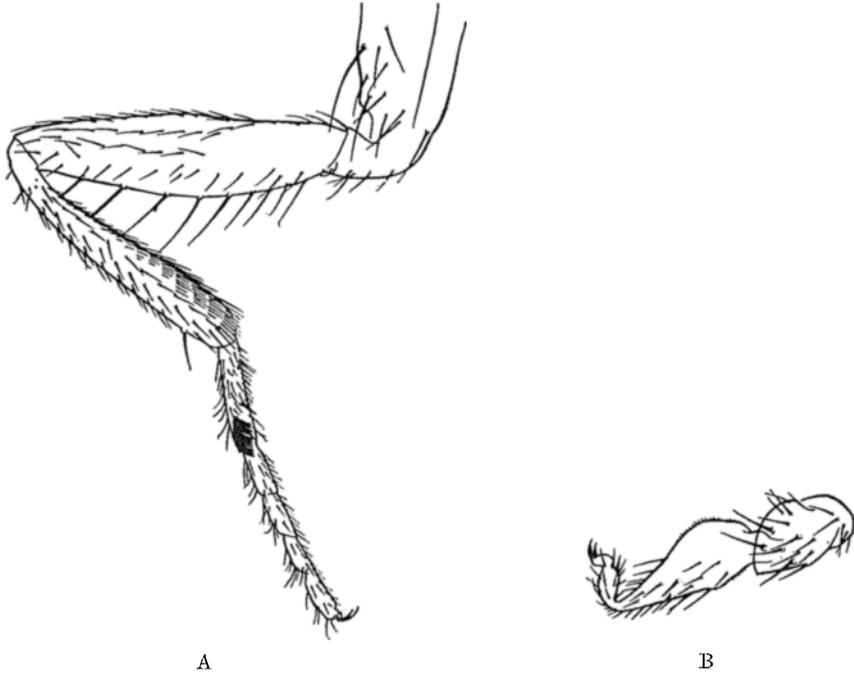


Fig. 2A, B. Foreleg and antenna of ss^{a40a} , treatment 1 (tarsal grade 5, arisal grade 8).

It can be seen from Fig. 4 that in the higher grades of expression of those alleles which have strong effects on the legs as well as on the aristae, this leg effect is also expressed in the arisal leg. It had already been shown (Waddington, 1940) that the arisal leg is affected like a normal leg by genes which alter the segmentation of the tarsus, such as *dachs*, *four-jointed*, etc. The question was raised at that time whether the aristapedia alleles can not only cause the arisal bud to develop into a tarsus, which involves some increase in size, but then also cause the abnormalities in segmentation, which involve a reduction in size. In the 'strongest' allele studied at that time, *aristapedia-Spencer* (ss^{aSp}), such an effect was not certainly verifiable, but in ss^{a40a} and ss^{aSnB} , both of which have very strong effects on the legs, it is clear that the arisal tarsus is reduced in flies with the strongest expression as compared with those with a weaker expression.

The biochemical mechanism of action of the temperature effect is still unknown. In a small series of experiments (made by Miss J. Macdonald) it was shown that the expression

of ss^{aB} is affected in the usual way by temperature in stocks which are being cultivated in sterile conditions. The effect cannot, therefore, be indirectly exerted through an influence on the yeasts and other microflora of the culture, which make up the principal nutrition of the larvae.



Fig. 3 A, B. Foreleg and antenna of ss^{a10a} , treatment 5 (tarsal grade 3, aristal grade 8).

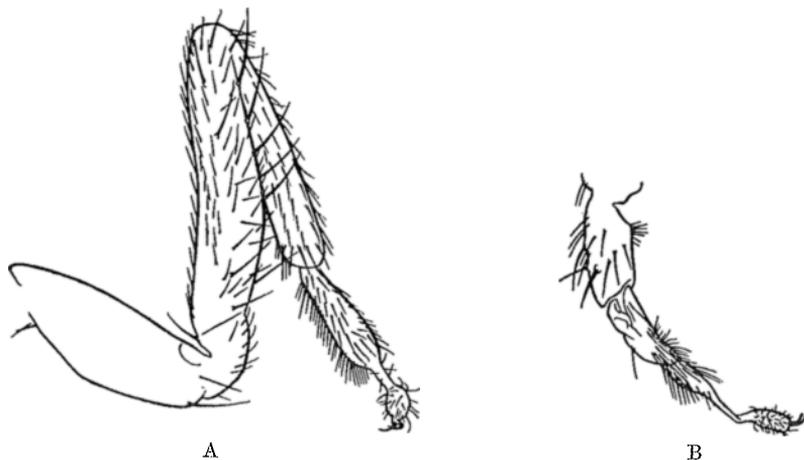


Fig. 4 A, B. Foreleg and antenna of ss^{aSnB} , continuously at 18° C. (tarsal grade 1, aristal grade 9).

DISCUSSION

It is clear that the aristapedia alleles show somewhat greater similarity in action than had been previously realized, since all those studied are capable of affecting both the antenna and the tarsus. Probably a full inspection would show that they also resemble one another in the other effects of the locus, for instance, on the length of the macrochaetae. The

dominant ss^{aR} described by Le Calvez (1948*a, b*) may be an exception, since it is associated with a chromosome rearrangement, and some of the numerous secondary effects described may not be due to the ss^a locus.

As regards any one type of effect, the alleles behave, at least roughly, as if they differed only quantitatively. Waddington (1940) figured a number of arisal legs from heterozygotes between the alleles ss^a , ss^{aSp} and ss^{aB} , and showed that the heterozygotes were more or less intermediate between the two homozygous types. However, if attention is paid to both the antennal and the tarsal effects, it seems impossible to attribute the differences between the alleles to such a simple quantitative relationship. By no means is it possible to convert an ss^{aB} fly into a form typical of ss^a , since if, by suitable temperature treatment, the antennal effects are made similar, the legs in the ss^{aB} flies will be much more strongly affected than they are in the ss^a animals. Again, if ss^{a40a} and ss^{aB} flies have similar legs, the former will have much more abnormal aristae. Thus it appears that each of the alleles ss^a , ss^{aB} and ss^{a40a} has a characteristic pattern of effect; ss^{aSnB} , which is not certainly a different allele from ss^{a40a} , may on the present data resemble it in pattern and differ only in intensity. Probably ss^{aSp} , which was studied earlier by Waddington (1940), closely resembles these two. Whether the other alleles of aristapedia fall into these groups or have other characteristic patterns of effect cannot yet be stated.

There appear to be two main ways of accounting for the existence of such different patterns of effectiveness. Either one can suppose that the different alleles produce qualitatively different (though presumably related) 'aristapedia substances', to some of which the arista is more sensitive than the legs, while for others the sensitivity is reversed. Or one might suppose that the alleles produce quantitatively different amounts of one and the same 'aristapedia substance', but at the same time alter the sensitivity of the legs and antennae to it. There seems little doubt that an important role is played by tissue sensitivity to some disturbance caused by the aristapedia gene, since threshold phenomena are clearly involved. Thus it was shown previously (Waddington, 1940) that, although the legs of ss^a flies are quite normal at 25°, there is a subthreshold sensitizing effect which becomes manifest if the ss^a flies are made simultaneously homozygous for other genes which affect the legs, such as *dachs* or *four-jointed*. There is, however, no direct evidence that there are differences in thresholds between flies carrying the various alleles, and the second hypothesis mentioned above seems a somewhat artificial one. Even if it were accepted, it still remains impossible to reduce the difference between the alleles to a merely quantitative relationship; all that would be involved would be that the non-quantitative aspect of the differences is shifted from the production of hypothetical active substances to the production of other substances which condition the tissue sensitivities.

The nature of the differences between alleles which have been shown to differ qualitatively cannot, of course, be stated at all definitely in our present state of ignorance about the gene. It may, however, be pointed out that changes in the molecular architecture of enzymes (e.g. changes in the carriers of the prosthetic groups) are known in some cases to alter differentially the effectiveness of the enzyme in promoting the various reactions which it may catalyse; and this may give a hint as to the relation between the different aristapedia alleles.

It is worth noting that although the moderately strong aristapedia effects involve an increase in the size of the arista, and thus at some stage an increased growth rate, they simultaneously involve a reduction in size of the tarsus; and in the extreme grades,

a similar reduction affects the arisal tarsus also. It therefore seems unlikely that the primary effect of the aristapedia locus can be considered as a mere stimulation of growth, as Goldschmidt (1938) and Vogt (1946*b*, 1947) have suggested. Even on the legs and antennae (omitting any consideration of the macrochaetal effects) the action of the locus must be complex, involving both the conversion of the arista to a tarsus and the distortion of tarsal development. It is perhaps simplest to suppose that these two gene actions take place successively, the arisal effect happening the earlier, so that by that time the gene is active in the tarsus, the developmental fate of the arista has already been determined. The concept of the time of gene action is, however, a very difficult one, and it is not easy to obtain unequivocal evidence about it. It might well be that the aristapedia genes first produce some substance in the antennal bud, which is such that if a later action of the same gene causes this bud to develop into a tarsus, that tarsus will be distorted. An accurate determination of the sensitive periods of the two effects might possibly give some indication of which effect occurs first, but even such evidence if it were available (which it is not) would probably be open to several interpretations.

SUMMARY

1. Larvae homozygous for several alleles of aristapedia (ss^a , ss^{aB} , ss^{a40a} and ss^{aSnB}) were transferred for three days to 18° C. after being kept at 27° C. for 12, 24, 36, 48 or 60 hr. from laying. They were then scored for the grade of transformation of the arista into a tarsus and for the distortion of the normal tarsus. In all cases both the abnormalities were increased by cold treatments given after 48 or 60 hr.

2. The patterns of effect were similar in ss^{a40a} and ss^{aSnB} , both of which always have a strong arisal effect and in the higher grades a strong tarsal effect; ss^{aSnB} is always stronger than ss^{a40a} under the same conditions.

3. A different pattern is characteristic of ss^a , which has a strong arisal effect and a weak tarsal effect. The pattern is different again in ss^{aB} , which at high temperature has a weak effect, and at low temperature a moderate effect, on both aristae and tarsi.

4. The existence of these different patterns of effect shows that the alleles are not related in a simple quantitative manner, but differ qualitatively.

5. In flies homozygous for alleles which have strong effects on both organs (i.e. ss^{a40a} and ss^{aSnB}) the arisal tarsus may be stunted just as is the leg tarsus. This demonstrates that the effect of the gene is not produced by a simple growth stimulation.

REFERENCES

- BUZZATTI TRAVERSO, A. (1947). *Riv. Sci. Nat. Natura*, **38**, 45.
 GOLDSCHMIDT, R. (1938). *Physiological Genetics*. New York and London.
 LE CALVEZ, J. (1948*a*). *Arch. anat. micr. morph. exp.* **37**, 50.
 LE CALVEZ, J. (1948*b*). *Bull. Biol.* **82**, 97.
 MULLER, H. J. (1932). *Proc. 6th int. congr. Genet.* **1**, 213.
 SPERN, C. (1930). *Multiple Allele*. Berlin: Handb. Vererbungswiss.
 VILLEB, C. A. (1943). *J. Exp. Zool.* **93**, 75.
 VOGT, M. (1946*a*). *Biol. Zblt.* **65**, 238.
 VOGT, M. (1946*b*). *Z. Naturforsch.* **1**, 469.
 VOGT, M. (1947). *Experientia*, **3**, 156.
 WADDINGTON, C. H. (1940). *Growth*, Suppl. vol. p. 37.