Experiments on canalizing selection*

By C. H. WADDINGTON

Institute of Animal Genetics, Edinburgh, 9

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INTRODUCTION

It is a platitude to point out that natural selection is not only responsible for the elaboration of evolutionary novelties, but that it has an even more universal function in the preservation of normality by the elimination of aberrant individuals. This second type of action has been referred to as stabilizing selection (Schmalhausen, 1949). This may operate in two rather different ways (Waddington, 1940, 1957). In the first place, natural selection will tend to eliminate those alleles which in the normal environment cause the development of abnormal phenotypes; this may be called normalizing selection. A rather different type of natural selection will tend to remove those alleles which render the developing animal sensitive to the potentially disturbing effects of environmental stresses, and will build up genotypes which produce the optimum phenotype even under sub-optimal or unusual environmental situations. This type of selection has been referred to as 'canalizing selection', since it brings it about that the epigenetic systems of the animals in the population are canalized, in the sense that they have a more or less strong tendency to develop into adults of the favoured type.

It is well known to all geneticists that different strains differ in their sensitivity to environmental influences. A genetic variability on which canalizing selection could operate must therefore exist. There has, however, been rather little experimental work directly concerned with investigating the response to canalizing selection and the magnitude of the effects which can be produced, although many instances in which canalizing selection was actually practised could probably be found by a careful search of the older literature, particularly that of Plant Genetics.

Among the newer studies on the quantitative genetics of animals, the experiments of Mather (1955) and Tebb & Thoday (1954) perhaps come closest to this aim. They investigated the effects of selection and various other genetic procedures on the difference in the number of sternopleural bristles on the two sides of a Drosophila, and demonstrated that the genotype may have an effect on the magnitude of this difference. It may be presumed that the asymmetry between the two sides of the individual arises in response to slight differences in conditions between the two regions of the developing animal. Nothing, however, is known about the nature of these presumed differences during development. Reeve &

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* A preliminary communication of some of these results has been made in *Nature, Lond.,* **183,** 1654 (1959).

Robertson (1954) studied a situation which is perhaps of a similar kind; namely, the differences in the bristle numbers on adjacent abdominal segments in *Drosophila*. They have spoken of these differences as arising from 'intangible accidents of development'. It is, indeed, not entirely clear that either type of difference has any definite ascertainable cause. It is possible that some developmental processes have an inherently stochastic character, and the divergences between the various elements of an essentially repetitive or symmetrical system may probably best be compared to 'noise', in the information-theory sense (Waddington, 1957). It is at any rate not at all clear that the genetic control of such a character can be taken as a guide to the nature of the genetic effects exerted on responsiveness to specific environmental stresses.

Falconer (1957) and Falconer & Robertson (1956) have also carried out experiments which involved selection of a type closely connected with canalizing selection. The former, working on *Drosophila*, selected those individuals whose bristle number on two abdominal segments lay closest to the mean of the population. Falconer & Robertson applied selection in mice for individuals whose weights were closest to the mean. In both these experiments the selection would have involved both the normalizing and the canalizing aspects of stabilizing selection, but in neither case was any definite environmental stress applied, and the relative importance of the two types of selection remains unknown. In the event, neither of the experiments revealed any marked response to the selective pressures applied, which, however, were rather small.

For these reasons it has appeared desirable to carry out an experiment designed to show whether canalizing selection can be actually effective in reducing the amount of phenotypic variation produced by a definite and known environmental stress.

MATERIALS AND METHODS

All the experiments were made on *Drosophila melanogaster*. The development of normal wild-type individuals of most stocks of this species is already rather highly canalized, so that the easily applied environmental stresses produce rather little phenotypic divergence from normality. It might therefore be rather difficult for a selection experiment, lasting a comparatively few generations, to produce marked changes in canalization, and in particular increases of it. The experiments were therefore made on mutant stocks in which, as is well known, the phenotype is often much more variable and sensitive to environmental influences than is that of the wild type. Four mutant types have been studied: Bar, dumpy, cubitus-interruptus, aristopedia-Bridges.

1. *Bar*. The number of facets in the eye is markedly reduced, the effect being stronger in the homozygous females than in the hemizygous males. The number of facets produced by a given genotype is known to be greater at a low temperature than at a higher one. The facet numbers can be counted only with some difficulty in the living fly, and estimations of average facet numbers in a population were therefore made by counts on eyes prepared after death. The parents for carrying

on the selection lines were selected on the basis of estimated eye sizes, as judged by inspection with a dissecting binocular.

2. *Dumpy*. This gene produces a shortening of the wing which is more extreme at high temperatures that at low ones. The effect was estimated by measuring the wing length from the base of the wing to the most extreme point of the rounded tip.

3. *Cubitus-interruptus*. The gaps which this gene produces in the posterior longitudinal vein are more extreme at low than at high temperatures. They were estimated in terms of an arbitrary series of grades founded on that used by Stern & Schaeffer (1943) (see Waddington, Graber & Woolf, 1957). In the figures given in this paper the numerical indices attached to the grades are one less than those used in the paper by Waddington, Graber & Woolf; that is to say, the wild type scores as 0 and the most extreme type as 6. These grades are of course quite arbitrary and can be used only for comparative purposes. The stock employed was a multiple recessive stock containing $ci\ gvl\ ey^R\ sv^n$.

4. *Aristopedia-Bridges*. This is a fairly weak allele of the well-known gene which tends to convert the aristae into leg-like appendages. The strain used was extracted from the F_2 of a cross between the aristopeida stock maintained in the laboratory and an Oregon-R wild type. The effect is more extreme at low than at high temperatures. It was estimated by a series of arbitrary grades, similar to that employed by Waddington & Clayton (1952). Among the more extreme modes of expression there are several in which the size of the aristal organ becomes reduced. In assigning numerical values to the grades these have been given relatively low values, similar to that of phenotypes in which only part of the arista has been converted into a leg-like structure. It is, however, by no means certain that this is the correct way of envisaging the situation. One of the effects of aristopedia is to cause reductions in the leg, and it may be that these reduced aristal organs really represent an extreme grade in the expression of the genic action, in which the arista is first converted into a leg and then reduced. If that were the case, the reduced organ should perhaps have a higher numerical index rather than a lower one corresponding to its size. It is unfortunate that, as it turned out, a considerable proportion of the populations kept at low temperatures had phenotypes within this range. As will be seen later, the selection practised had little effect in altering the average phenotype of these cold-temperature populations, and it may well be that owing to the uncertainties of the grading system very little selection was in fact exerted on them.

Three types of experiments have been carried out. The first two were both varieties of what may be called alternate selection. In these the strain was kept for one or more generations at one temperature, then moved to the other temperature for one or more generations, and then back again to the first set of conditions, and so on alternately. Breeding always involved selecting against the known environmental effect of the temperature. That is to say, if, as in Bar, a low temperature tends to cause an increase in the number of eye facets and a higher temperature a reduction in facet number, one would select from a population

reared at a low temperature those with the smallest facet numbers, and from a population bred at a high temperature one would select those with the largest number of facets. The two experiments carried out according to this plan differed only in that, in the first of them, one generation at a low temperature of 18° C. alternated with two generations at the high temperature of 25° C., while in the second experiment only one generation at the high temperature followed one generation at the low. In a variant of the first type of experiment two generations at 25° were run simultaneously with one at 18°, and individuals from the second 25° generation were then crossed with those from the 18° generation to produce two new selected lines, one of which was again given two generations at 25° while the other had one generation at 18°, when the process was repeated.

A very different and, as it turned out, much more effective type of selection was applied by means of family selection. In this, a series of pair-matings were set up, and from each pair of parents a number of offspring were reared at 25° and a number at 18°. Family averages of the difference between the phenotypes at these two temperatures were then ascertained by measuring a certain number (usually twenty) of the offspring at each temperature. Selection was made on the basis of these family averages, lines being carried on by breeding together sibs from the families which exhibited the least environmental sensitivity and in which the difference between the offspring at the two temperatures was smallest.

RESULTS

1. *Alternate selection*

The results of the experiments carried out with Bar, dumpy and cubitus-interruptus are given in Tables 1 to 3. It will be seen that there was considerable progress in reducing the environmental sensitivity of the Bar stocks. In dumpy and cubitus-interruptus only rather slight progress was made. This result may be partly due to the necessity to use, in these two stocks, a somewhat inadequate system of scoring by arbitrary grades. The imperfections of this system probably reduce the amount of selection that can be exerted, and it probably reflects only very inaccurately the actual situation in the stocks which eventually arise. In spite of this, some slight reduction in environmental sensitivity has been achieved in nearly all cases. The magnitude of the effects, however, is so slight that it could hardly be taken to be sufficient to establish the reality of the process.

2. *Family selection*

Family selection experiments were carried out with Bar and with aristopedia-Bridges. The results are shown in Tables 4, 5 and 6. In both cases very marked reductions in environmental sensitivity have been achieved.

In these experiments selection was carried out on the basis of the average magnitude of the difference between the high- and low-temperature sibling groups. No attention was paid to the absolute magnitudes of the phenotypic indices. In

Table 1. *Bar. Mean facet number per eye (two sides averaged)*

	Generations									
	1	2	3	4	5	6	7	8	9	10
Temperature	25	25	18	25	25	18	25	25	18	25
♂	91	82·6	222·8	100·0	117·7	191·6	91·0	101·0	182·3	90·0
Difference, 18°—25°			140·2	122·8		73·9	100·6		81·3	92·3
♀	46·9	51·8	160·1	66·8	56·7	107·3	61·0	69·3	95·5	58·4
Difference, 18°—25°			108·3	99·3		50·6	46·3		26·2	37·1

	Generations							
	11	12	13	14	15	16	17	18
Temperature	25	18	25	25	18	25	25	18
♂	127·9	183·8	116·0	106·2	196·0	122·8	110·8	204·8
Difference, 18°—25°		55·7	67·8		89·8	73·2		94·0
♀	73·7	102·5	68·0	63·3	103·4	71·0	71·5	102·7
Difference, 18°—25°		28·8	34·5		40·1	32·4		31·2

the case of Bar this has resulted in the production of a relatively temperature-insensitive eye of a size intermediate between that which was characteristic of the low- and high-temperature populations at the beginning of the experiment. Some representative figures on this point are given in Table 5. With aristopedia the result was rather different (Table 6). The reduction in temperature sensitivity has in essence been brought about by increasing the abnormality of the high-temperature population, in which initially the gene was rather weakly expressed. There has been scarcely any accompanying reduction in the expression in the low-temperature group. As was pointed out in the section on methods, this is probably a consequence of the imperfections of the scoring system, which seems likely to be particularly unreliable in discriminating between the various higher grades of expression, so that it seems probable that in fact very little selection of any kind was actually exerted on the low-temperature populations.

It may appear from Table 4 that the response to selection has been greater than the selection-pressure applied, since in many instances the later generations show a smaller average family difference than that found in the family which was selected in the immediately preceding generation. However, the figures given in brackets in that table, which record the difference between the 25° and 18° groups of the families selected, do not tell the whole story, since selection was also practised *within* the families, by selecting individuals which showed little effect of the temperature treatment. It is therefore impossible to give any accurate analysis of the rate of response to the selection-pressure. The bracketed figures are given merely to show that the response cannot be considered a slow one.
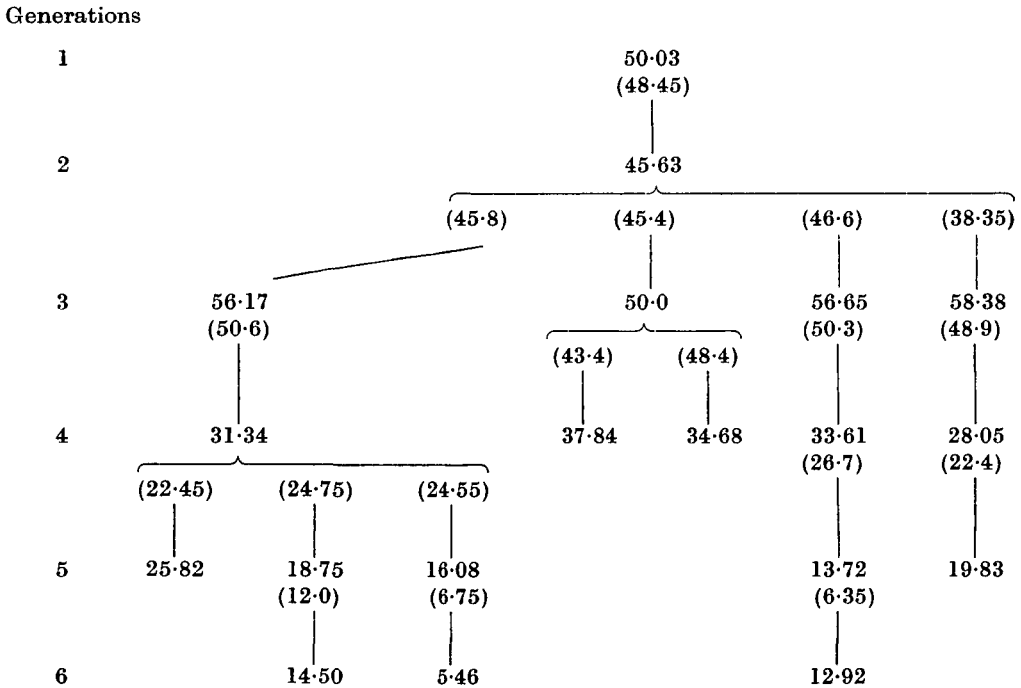
Table 2. *Dumpy. Wing lengths (two sides averaged)*

	Generation											
	1	2	3	4	5	6	7	8	9	10	11	12
Expt. 1 Temperature	25	25	18	25	25	18	25	25	18			
Wing length ♂	55·8	53·4	70·4	55·3	56·5	76·35	57·65	61	78·4			
Difference, 18°—25°		17·0	15·1		19·85	18·7		17·4				
Wing length ♀	58·8	56·95	75·65	59·9	63·3	76·8	62·1	63·1	80·1			
Difference, 18°—25°		18·7	15·75		13·5	14·7		17·0				
Expt. 2 Temperature	25	18	25	18	25	18	25					
Wing length ♂	52·8	75·35	58·5	76·15	58·6	77·65	61·45					
Difference, 18°—25°	22·55	16·85	17·65	17·55	19·05	16·2						
Wing length ♀	55·05	77·45	61·75	74·45	62·7	78·6	63·95					
Difference, 18°—25°	22·4	15·7	12·7	11·75	15·9	14·65						
Expt. 3 Temperature	25	25	18	25	25	18	25	25	18	25	25	18
Wing length ♂	58·1	57·8	76·45	56·1	60·15	75·45	60·2	60·1	76·25	61·5	63·1	79·35
Difference, 18°—25°		18·65	20·35		15·3	15·25		16·15	14·75		16·25	
Wing length ♀	59·48	59·4	77·45	60·15	60·85	74·45	59·75	64·0	79·55	64·1	63·5	79·5
Difference, 18°—25°		18·05	17·3		13·6	14·7		15·55	15·45		16·0	

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Table 3. *Cubitus-interruptus. Mean grade (two sides averaged)*

	Generations														
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Temperature	25	25	18	25	25	18	25	25	18	25	25	18	25	25	18
♂	138	127	202	151	150	212	140	134	208	140	159	260	156	140	196
Difference, 18°−25°		75	51		62	72		74	68		51	54		56	
♀	93	96	182	98	116	199	127	83	170	113	132	151	133	130	194
Difference, 18°−25°		86	84		83	72		87	57		19	18		64	

Table 4. *Bar. Facet number in females. Pedigree of family selection experiment. The figures give the differences between the means of the family-samples reared at 25° and at 18°. The plain figures give the average of the differences of all families tested; the figures in brackets the difference in the family selected to carry on the line*

Generations

1		50·03 (48·45)
2		45·63
3	56·17 (50·6)	(45·8) (45·4) (46·6) (38·35) / 50·0 56·65 (50·3) 58·38 (48·9)
4	31·34	(43·4) (48·4) / 37·84 34·68 33·61 (26·7) 28·05 (22·4)
5	(22·45) (24·75) (24·55) / 25·82 18·75 (12·0) 16·08 (6·75)	13·72 (6·35) 19·83
6	14·50 5·46	12·92

In view of the interest which has been taken in asymmetry as an indication of developmental stability, it seemed interesting to inquire whether successful canalization against the effect of temperature was accompanied by a greater stability against the unknown environmental variations to which asymmetry may hypothetically be attributed. The asymmetry of facet numbers was therefore estimated, the measure used being the difference in the logarithms (to base 10) between the larger and the smaller of the two eyes. As can be seen from Table 7,

there was little alteration in the asymmetry during the course of selection; in the twenty-three families measured after selection, the asymmetry at both temperatures was slightly less than it had been in the set of pair-matings from which the

Table 5. *Bar. Facet numbers in females in unselected controls, and in three families resulting from selection*

	No. of Families	Average family mean, at 18° C., and s.e.	Average family mean, at 25° C., and s.e.	Difference, family means, 18° C. and 25° C.
Unselected	9	156.25 ± 4.81	55.46 ± 0.81	100.80 ± 5.42
Selected 6a	8	106.02 ± 1.17	91.51 ± 1.67	14.50 ± 2.29
Selected 6b	7	100.76 ± 1.38	95.30 ± 1.70	5.46 ± 1.98
Selected 6c	8	111.39 ± 1.72	98.48 ± 2.67	12.92 ± 3.05

Table 6. *Aristopedia-Bridges. Family mean grades*

Generation	No.	18°	25°	Difference
0	10	14.01 ± 0.085	7.16 ± 0.215	6.84 ± 0.177
1	20	13.36 ± 0.130	7.46 ± 0.152	5.89 ± 0.157
2	20	13.74 ± 0.067	7.89 ± 0.141	5.90 ± 0.167
3	20	13.58 ± 0.062	7.39 ± 0.184	6.20 ± 0.20
4	20	13.36 ± 0.07	8.72 ± 0.13	4.65 ± 0.14
5	20	13.60 ± 0.071	10.50 ± 0.179	3.10 ± 0.190
6	19	13.44 ± 0.626	11.13 ± 0.286	2.31 ± 0.268

Table 7. *Mean asymmetry in facet number (log_e scale)*

Selected for	Progeny of	18° C.	25° C.
0 generations	20 pairs	0.102 ± 0.025	0.095 ± 0.018
0 generations	Foundation pair	0.115	0.069
6 generations	23 pairs	0.093 ± 0.026	0.081 ± 0.026

selection started, but the difference is small and non-significant. When one compares the families at the end of selection with the particular foundation pair-mating from which they were all derived, the asymmetry at 25° is found actually to have increased. One may conclude that selection for better buffering against the external temperature has had little or no effect on asymmetry.

DISCUSSION

The family selection experiments, particularly that with Bar, provide a very clear example of the effectiveness of canalizing selection. In the unselected population the eyes of individuals reared at low temperatures have about three times as many facets as those reared at 25°, while by the end of the selection experiments the difference has been reduced to about 10%.

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This reduction in environmental sensitivity has occurred in an epigenetic system which is abnormal in the sense that it is affected by an unusual allele, namely, the dominant mutant gene Bar. There seems no reason to doubt, however, that similar processes of selection would be effective in reducing the environmental sensitivity of a population in which no obviously abnormal allele was present. The array of genotypes which fall within the broad category of the wild type all give rise to epigenetic systems which are, in general, rather insensitive to external stresses, but it seems inevitable that amongst them there must be some genotypes which provide better developmental buffering than others. It was not by any means obvious, however, that such an abnormal developmental system as that resulting from the Bar mutant could be brought to a condition of such temperature-insensitivity as we have found. Since canalizing selection, operating through families, has been so extremely effective on this abnormal developmental system after only five generations, one is left with a feeling of considerable confidence as to its powers when acting on more normal populations.

The comparative failure of systems of alternate selection is perhaps not very surprising. When in an 18° population of Bar one selects the individuals with the smallest eyes as parents of the next generation, one is, of course, not only exerting canalizing selection in favour of genotypes which are insensitive to the temperature effect, but one is also applying progressive selection in favour of genotypes which would tend to produce a small number of facets under any environmental circumstances. In the next generation, reared at 25°, selection for large eyes would again favour well-canalized genotypes but would now also involve a progressive selection of the opposite kind to that in the previous generation, since it would favour genotypes which tended to produce large facet numbers under all circumstances. The canalizing selection would only be effective in so far as the two opposite types of progressive selection cancelled each other out, and in proportion to the importance of canalizing factors as opposed to facet-increasing or facet-reducing factors in determining which individuals will be selected. There is no way of telling *a priori* which will be the more important elements in the situation. The fact that in these experiments there was in several cases an immediate slight increase in canalization, but that the rate of progress was not maintained thereafter, suggests that the presence of alleles of different quantitative effectiveness has made it very difficult by this procedure to concentrate alleles whose effect is primarily on environmental sensitivity.

The mathematical theory for selection for canalizing genes has never been worked out. It is, however, easy to show that selection which operates only against the phenotypic effect of a single environment, such as that which was exerted in the alternate selection experiments, would often tend to concentrate quantitatively-acting genes rather than canalizing genes. Consider, for instance, a population which contained a relatively rare allele a whose effect in all environments is to reduce the size of a given phenotypic measure. Let the frequency of a be p. Further, suppose the population contains an allele b with a frequency of p and with the effect that the homozygote bb is less environmentally sensitive. Now,

suppose the population to be in an environment which tends to increase the phenotypic measure, and let selection be applied against this environmental effect. We will suppose that the selected group includes all the genotypes containing aa, and that the reduction in phenotypic sensitivity brought about by b is such that the genotypes $Aabb$ would also fall within it. Then in the next generation

$$p_1 = \frac{2p^2 + 2pqP^2}{2p^2 + 4pqP^2} = \frac{p + qP^2}{p + 2qP^2}$$

and

$$P_1 = \frac{2p^2(P + PQ) + 4pqP^2}{2p^2 + 4pqP^2} = \frac{pP + 2qP^2}{p + 2qP^2}.$$

Thus, for the proportional rates of increase of a and b we shall have

$$\frac{p_1 - p}{p} = \frac{q[p + P^2(1 - 2p)]}{p(p + 2qP)} \quad \text{and} \quad \frac{P_1 - P}{P} = \frac{2qP(1 - P)}{p2qP}.$$

The 'quantitative gene' a will increase proportionately faster than b if

$$\frac{p + P^2(1 - 2p)}{p} > 2P(1 - P),$$

i.e.

$$P^2 > p(P - Q).$$

It is clear that, with appropriate values of the frequencies, either the quantitatively-acting or the canalizing genes will increase the more rapidly. Without some prior knowledge of the relative frequencies of genes of these two kinds, one cannot predict what the outcome will be.

The lack of response of asymmetry to selection which was effective in reducing sensitivity to the particular environmental variable of temperature is worth noting. But it should occasion no surprise. Reasons have been offered previously (Waddington, 1957) for expecting differences between comparable regions within a single individual body to be controlled by genes which are not the same as those which affect the reaction of the body as a whole to the external environment.

SUMMARY

1. Mutant stocks whose phenotype is affected by temperature were kept at 18° and 25° C., and selection was applied in an attempt to reduce the magnitude of the phenotypic difference caused by the temperatures. The stocks used were Bar, dumpy, cubitus-interruptus and aristopedia-Bridges.

2. When the stocks were kept for one or more generations at one temperature, then transferred for the next generation or two to the other, and so alternately, selection applied against the phenotypic effect of the temperature was only slightly effective in reducing the differences between the high- and low-temperature phenotypes. It is suggested that this was due mainly to the fact that such selection would be expected to operate on quantitatively-acting genes as well as on genes controlling developmental buffering.

3. With family selection, in which the offspring of a pair-mating was divided into two lots, one kept at each temperature, and selection was made on the basis of the differences in family means, progress was rapid. In the unselected Bar stock, the facet number at 18° was initially about three times that at 25°; after six generations of family selection the difference had been reduced to about 10%, the phenotypes at both temperatures being about intermediate between those seen at the beginning of the experiment. With aristopedia the reduction in the difference in family means was also striking, but in this case was achieved by increasing the abnormality of the 25° phenotype, and hardly at all by lowering that of the 18° population. It is pointed out that this is probably the result of an inadequate scoring system.

4. Although the selection in Bar stocks was effective in increasing developmental canalization against external environmental changes (temperature), it had little or no effect on the asymmetry of the facet numbers.

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