GENETIC RESPONSE TO SELECTION IN CULTIVATED PLANTS : GENE FREQUENCIES IN *PRUNUS AVIUM*

WATKIN WILLIAMS and A. G. BROWN

John Innes Horticultural Institution, Bayfordbury, Hertford, Herts

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I. PREMISES OF THE INVESTIGATION

THE study of allelic frequencies in species of plants with a long history under cultivation affords an excellent opportunity for understanding the interplay of genetic changes which follow evolutionary advance under selection. On the more immediately obvious theoretical grounds, perhaps the most significant single feature to be expected from adaptive advance is a reduction in total genetic variability as represented by the frequency of alternative alleles available at a single locus. In the absence of equilibrating forces, such as heterozygote superiority, or of disturbing linkages with lethal genes which are a constant feature of many types of polymorphism, and excluding cyclic changes in the adaptive values of homozygotes, directional selective trends are expected to achieve genetic fixation of all the loci which affect the adaptive value of the phenotype. In respect of mutant alleles at loci neutral to adaptation, if any exist, their rate of spread would be so slow that little or no variation would ever develop at the locus (Fisher, 1930). Where selection pressure is high, and the state of sexual isolation from divergent populations undergoing adaptation in different directions is almost complete, the amount of allelic variability remaining in populations at equilibrium is expected to be at the extremely low level which only the rate of mutation can sustain.

The ruthless suppression of unfavourable genes and the high degree of reproductive isolation which are the rules of the plant breeder, and the dissection of the population into a series of small, discontinuous, breeding units in the search for maximum phenotypic expression and uniformity, represent a pressure of selection unequalled in nature, except in cases of dominant lethals. It is in cultivated plants and domesticated animals therefore, that one would expect the minimum of allelic variation in respect of all loci irrespective of whether they are adaptive or neutral. This is the thesis that has led Harland on to the notion of genetic erosion during domestication.

A reduction in the available variability is unavoidable under conditions where but a few superior genotypes become the founders of a large specialised population. The importance of the "founder principle" in evolution has been recognised and elaborated notably by Mayr (1942), and its profound effect on improved populations of plants in cultivation is inescapable. Nevertheless the evidence to be presented here shows clearly that the erosion of genetic variability

is not the property of selection at the loci under study. On the contrary the selective advances which have occurred since the domestication of the sweet cherry, appear to have necessitated the conservation of variability through heterozygosis even in what appear superficially as neutral or deleterious genes.

The sweet cherry is an outbreeding diploid with 16 chromosomes and has had a long history under cultivation in Europe and the Near East. Selection under cultivation has probably existed for over 2000 years. In addition, some varieties have had a continuous recorded history for upwards of four centuries. The variety Noble, for example, which is a well-known form in present-day orchards is one which was first described in England in 1611. Under such circumstances only continuous selective trends over very long periods need be considered in relation to frequency levels of allelomorphs at a given locus. Cyclical changes in the environment even if modulated over many years could not be expected to operate in this material. Throughout the cultivated history of the plant selection has been operating for polygenic characters such as season, size and quality of fruit. Fruit colour, which is partly under oligogenic control, has at best probably been subject to direct selective pressure of a very low order. We are therefore concerned here with intensive selection for polygenic complexes seemingly independent of oligogenic variation. The fate of variation at loci with major effects, when selection is for quantitative characters, should prove informative not only in relation to purely plant breeding problems, but also for the understanding of the broader evolutionary issues of polymorphisms and of what Lerner (1953) has termed homeostasis.

Three major gene loci in the European population of cultivated sweet cherry will be considered in this account. They comprise the S locus governing incompatibility, the b locus controlling pigmentation of the sap in the ripe fruit, and the a locus at which albinism is expressed by the homozygous recessive. Information is at present accumulating on a fourth locus where the recessive allele expresses dwarfism, but the data, although pointing towards the same general conclusions are not sufficiently exhaustive to be presented here. All the evidence available indicates that the three loci are independently inherited so that the effects of selection can be studied on sections of three different chromosomes out of the haploid set of eight.

2. FREQUENCIES OF S ALLELES

A total of 125 cherry varieties have been used in tests at the John Innes over the last thirty years, and a total of six incompatibility alleles have been definitely established in nine groups of varieties. Three other definite groups of varieties are known but their precise allelic constitution has not been determined. A thirteenth group has been formed for convenience, containing an assemblage of 20

varieties all of which are compatible with the other 12 groups and most of which are compatible with one another. This is therefore a group of groups, each of the true groups containing but one or, at best, a few varieties. The frequencies of the alleles represented in these latter varieties must necessarily be very low.

The allelic composition of varieties in the nine groups where precise determination of the alleles have been made is as set out in table 1.

Of the 65 varieties represented in the table, 42 or 65 per cent. possess the S₃ allele, and the most successful allele in combination with S₃ is S₁. In this connection it is interesting to note that S₁ is itself the second most successful allele of the series, and that S₂

TABLE 1
*The frequency of the alleles S₁-S₆ in 65 varieties
of sweet cherry*

	S ₃	S ₁	S ₂	S ₄	S ₅	S ₆	Gene frequency
S ₃	...	15	10	7	3	7	0.323
	S ₁	...	13	4	0	0	0.246
		S ₂	...	0	2	0	0.192
			S ₄	...	4	0	0.115
				S ₅	...	0	0.069
					S ₆	...	0.054

which also makes a successful combination with S₃, is likewise successful when combined with S₁. All the remaining combinations, excluding those with S₃, appear to have a decided disadvantage. Calculations of expectation in the various genotypic classes are not very helpful in this case, as the estimated gene frequencies have to be calculated from the present population and not from the natural ancestral population from which the material arose. The calculation of Chi is therefore biased in favour of agreement with the observed distribution. The value of χ^2 after correction of values for the obligate absence of homozygotes, gives a value of 4.58 (5 D.F.) which does at least tell us that no major disruptive forces are at work affecting the viability of the genotypes.

That S₃ has a strong selective advantage in the cherry can however be supported by a totally different kind of evidence which is not dependent on the initial frequency of the various alleles at this locus. In the course of cherry breeding at the John Innes, Crane and Brown

have selected 9 seedlings which were considered an improvement on existing varieties. It is not irrelevant to this discussion to state here that seven of these cherries are of undoubted merit and have been enthusiastically received by the industry. These seedlings and the genotypic constitution of their parents are set out in table 2.

It is seen from the table that eight out of the nine selected seedlings contain S₃. Merton Glory—the only exception—had no S₃ in its pedigree. In seven out of the eight selections containing S₃ the selector had a choice of either selecting or rejecting S₃. The probability of selecting S₃ by chance in these eight seedlings is therefore $(\frac{1}{2})^7$ or 0.008. This level of probability is far too low to be ascribed to chance, and offers strong support that S₃ itself or the chromosome segment

TABLE 2

The genotypic constitution of selected cherry seedlings and their parents

Seedling	Seedling constitution	Parental constitution
Merton Bigarreau . .	S ₁ ·3	S ₁ ·2 × S ₃ ·4
„ Bounty . .	S ₁ ·3	S ₃ ·6 × S ₁ ·3
„ Favourite . .	S ₁ ·3	S ₁ ·2 × S ₁ ·3
„ Premier . .	S ₂ ·3	S ₃ ·4 × S ₁ ·2
„ Heart . .	S ₃ ·6	S ₁ ·3 × S ₃ ·6
„ Late . .	S ₃ ·4	S ₁ ·3 × S ₃ ·4
„ Glory . .	?	S ₁ ·4 × S _x ·y
No. 42 . .	S ₃ ·6	S ₁ ·3 × S ₃ ·6
No. 1464 . .	S ₂ ·3	S ₂ ·x × S ₃ ·4

carrying S₃ has some cryptic effect on economic characters in the cherry and therefore possesses a decided selective advantage.

3. FREQUENCY OF ALLELES AT THE “b” LOCUS

As already indicated the pigmentation of fruit sap in cherries cannot be regarded as entirely free from influences on selection, but under the conditions that have operated on this character, its effect is considered to be very slight. However, this point need not concern us here as the allelic frequency under consideration is not dependent on the operation of selective bias. The pigmentation of sap is governed by two alleles—pigmentation (the so-called black cherries) being dominant to non-pigmentation (white cherries). The recessive genotype can have a varying degree of pigmentation in the skin. The homozygous dominant and heterozygous genotypes are phenotypically identical, and so far genetic tests alone can separate the two pigmented classes. Of the 94 varieties that are or have been in the Bayfordbury collection, 36 per cent. are homozygous recessive. A selection from this collection, at random for all characters except for

their suitability as high quality parents in breeding has given the following analysis :

	Homozygous black (+ +)	Heterozygous black (+ b)	Homozygous white (bb)
Observed	2	30	19
Expected	7.8	24.2	19

$$\chi^2 = 5.9 \quad P \ 0.02-0.01$$

If this estimate of the frequency of the two alleles is approximately correct, this sample shows a significant excess of heterozygotes and a deficiency of homozygous dominants. The reported frequency of the recessive phenotype in various collections in different parts of the world have been very variable so that a reliable estimate is difficult to obtain. Further, any estimate within cultivated collections would be of doubtful value, because previous to the present century new varieties originated as chance seedlings from small and widely dispersed plantations when crossing with wild sweet cherries would be more than a possibility. Nevertheless for the 51 varieties reported here, almost all other estimates of the frequency would give a worse fit and the excess of heterozygotes over expected would be increased.

From all the evidence at our disposal there is no indication that there is linkage between the b locus and the incompatibility locus that would explain the deficiency of the homozygous dominant. In all crosses where such linkage might have been detected, *i.e.*

$$\frac{S_x B}{S_y b} \text{ or } \frac{S_x b}{S_y b} \times \frac{S_x B}{S_z b} \sigma$$

no deficiency of one of the classes has been observed. Further the distribution of colour classes with respect of the different S alleles gives no reason to suspect linkage. The similarity between these genotypic frequencies and those of balanced polymorphisms in the Lepidoptera, where one of the homozygotes is kept rare by close linkage with embryonic lethals, forces us to enquire whether such a mechanism might not be operating in the present material. The good agreement with the expected 3 : 1 ratio given by a large number of families in which "b" was segregating precludes this possibility.

We may therefore conclude with reasonable certainty that in the course of selection for quantitative characters in the cherry this seemingly neutral major gene has had a definite selective advantage when in the heterozygous condition. The question of whether one is dealing with the effects of this gene only, or whether it is the result of the co-operative effect of linked genes on a segment on which "b" acts as a marker does not materially alter this simple conclusion.

4. FREQUENCY OF ALLELES AT THE ALBINO LOCUS

Lastly we have to consider the state of variability at the albino locus. The albino factor in this species follows a pattern similar to that described in many species of plants, *i.e.* it is a lethal recessive having all the text-book characters of a major gene. Owing to the long generation time in the cherry only those characters which segregate in the F_1 can be effectively studied in any reasonable period of investigation. Fortunately albinism is one such character, and of 33 varieties which have been used in suitable cross-combinations 17 (51.5 per cent.) are heterozygous for albinism. This analysis shows that the recessive allele occupies 0.26 of the positions available at this locus in this population. The mutant allele being a completely recessive lethal would in the absence of selection achieve equilibrium at a level approximately equal to the square root of the mutation rate. If we are to consider that here we have an allele which is selectively neutral and which has achieved equilibrium, we must also be prepared to believe in a mutation rate from the wild type of the order of 10^{-1} . This becomes clearly an impossible situation, not only from our knowledge of mutation rates, but also from our experience of the stability of the wild type allele in heterozygous varieties which have been under continuous observation over the past three and a half centuries.

In this connection it is interesting to note that Noble, a variety described as originating in this country as far back as 1611 is heterozygous for albinism. Similarly Hedelfingen raised in Germany in 1850, Jaboulay raised in France (1822), Florence raised in Italy (1825), and Semis de Burr raised in the U.S.A. (1844) are all known heterozygotes, indicating that the mutant has had a wide distribution over a long period, and that its presence in high numbers in the present sample is not due to it being exclusively favoured in the English Collection.

In attempting to understand the distribution figures of the two surviving genotypes involving this pair of alleles we may consider two possibilities. Firstly, the present level of the mutant allele may have been conditioned by a process analogous to genetic drift, or more aptly perhaps to Mayr's Founder principle. This means that sometime during its evolutionary history, perhaps during its initial domestication, the population became reduced by selection to a very narrow basis of a few individuals which became the founders of the subsequent population, and that by this process, the allele for albinism achieved accidentally this relatively high frequency. This hypothesis has only one attribute—simplicity, and even if it were valid one would still have to command the assistance of directional selective trends to explain the situation. The minimum population size that could have founded the cultivated cherries is two, and of the four alleles carried at this locus by these parents it is unlikely that the recessive lethal was represented at more than 1. Thus we have at best an initial frequency of 0.25 maintained over 2000 years at a constant level in

spite of the rigorous elimination of the homozygous recessive genotype. Assuming a normal rate of mutation; merely to maintain a lethal gene at a level of frequency of 0.25 we need a sustained superiority of the heterozygote at a level of about 30 per cent. above the homozygous dominant genotype.

Thus we are forced, irrespective of the functioning of the "founder" principle, to consider the second possibility, namely, the selective advantage of the heterozygote. One has to conclude again therefore that a cryptic selective advantage belongs to the heterozygote of this lethal mutant under the selective pressure which is exercised in the quest for improved expression of quantitative characters of cherries. It is worth noting in relation to this conclusion, that in an intensive study of 75 lethal alleles in *Drosophila melanogaster*, at least three possessed survival advantages in heterozygotes (Stern *et al.*, 1952).

5. DISCUSSION

Before this analysis became possible no one would have suspected the three loci under consideration of having anything but a neutral effect on the desirable fruiting qualities in the cherry. In the case of the incompatibility alleles, the locus is forced by the breeding system into an obligate state of heterozygosis so that discussion here resolves itself around the choice of the effective combination of alleles rather than on the significance of heterozygosis *per se*. Schultz and Briles (1953) have shown the advantage of certain combinations of alleles governing blood groups in poultry where the direction of selection was for increased egg production. These workers also showed that advantageous combinations at one locus were favoured by the epistatic effects of favourable combinations at other loci. Again the discovery of Irwin and his collaborators (1952) of hybrid antigenic substances in some but not in other of their hybrids between species of pigeons and doves, indicates a chemical mechanism through which such combinations could offer advantages. But why should hybrid substances produced at a locus whose only observable expression is confined to the pollen tubes and the styles at reproduction, have any effect on the expression of quantitative characters of importance in a breeding programme? The answer could be pleiotropy or it could be linkage with position effects.

In assessing the likely role of these two possibilities one has to note firstly that the permanent state of heterozygosity and the extent to which allelic variation can develop at this locus afford unique opportunities for the accumulation of mutations on which selection could only act through the heterozygote. Any new mutation in the region close to the S locus would either have to be a dominant mutation (which is unlikely) or would have to possess advantage in the heterozygous condition before it could be used in selection. Therefore heterozygous advantage appears to be a *sine qua non* of linkage of favourable alleles with position effect close to the S locus.

It does not appear necessary on grounds of first principles to invoke linkage when we discover a locus doing something outside the neat role that macrogenetics would ascribe to it for the sake of retaining a "tidy string of beads". It is inconceivable, specific though gene action may be on the phenotype, that variation at any given locus is without effect on the general biochemical activity of the cell at many stages of developmental processes. One cannot expect a single gene substitution to affect an interdependent series of reactions at one point without affecting the whole series to a greater or lesser extent. In other words new gene substitutions under selection will need altered environments in which to function. These can be provided only by directional responses at other loci to provide the most suitable equilibria for the changed reaction levels. This seems to be the most likely explanation for the allelic frequencies at the S locus in cherries. We are dealing in effect with a correlated response of the genotype following selective changes in other characters—a remote but probably very important form of pleiotropy.

Finally, in respect of the loci for pigmentation and albinism it is difficult to avoid the conclusion that the heterozygotes are superior. In the case of the lethal for albinism it would be exceedingly tortuous to suppose that linked dominant mutants have appeared to enhance the spread of the lethal in the heterozygous condition. Such a development even if it occurred would surely be cancelled by similar mutants on the wild type chromosome before the spread would have proceeded very far. When considering the superiority of the heterozygote it is important to realise that maximal gene effects need not in all cases represent an optimal condition, and heterozygotes with reduced effect may therefore be nearer to the optimum level in any given circumstance. This is clearly seen in the sulphanilamide-requiring strain of *Neurospora* (Emerson, 1948) where the heterokaryon for the suppressor, owing to a reduction in the turn-over of para-amino benzoic acid gives a better growth response. This principle may well be applicable in cultivated plants where gene expression has been pushed to extremes in some directions, and where compensation in other directions is needed to preserve a normal functioning organism. This idea is not new, it has been conveyed in general terms as "integration" (Darlington (1939)), "internal" and "relational" balance (Mather (1943)), "stabilising selection" (Schmalhausen (1949)) and "developmental homeostasis" (Lerner (1954)). Integration in this material appears to be achieved through the heterozygote and not through genetic erosion and fixation. The process proceeds automatically and in parallel with selective advance. This being so, these examples prompt the suggestion that oligogenes play an important polygenic role in the expression of most characters. Indeed may not the basis of quantitative inheritance depend, partly at least, on the interplay of oligogenes?

6. SUMMARY

1. The frequencies of alleles at the loci which govern incompatibility, pigmentation of the fruit juice, and lethal albinism have been studied in cultivated varieties of the Sweet Cherry—*Prunus avium*.

2. Selection for quantitative economic characters has favoured heterozygotes of the S₃ allele at the incompatibility locus, the heterozygote for albinism, and the heterozygote for pigmentation in the fruit juice.

3. These results are discussed in relation to selective properties of apparently neutral, major gene loci, and to the advantages of heterozygosis.

4. The observation is made that the effect of some of the alleles in selection may be due to secondary pleiotropic action on polygenic characters. Pleiotropy in this instance may result from the effects of the alleles modifying the genotype as a whole.

5. The alternative hypothesis of close linkages with groups of polygenes affecting quantitative characters is considered critically, and it is suggested that the present result can be explained without invoking linkage effects.

7. REFERENCES

- DARLINGTON, C. D. 1939. *The Evolution of Genetic Systems*. Camb. Univ. Press, Cambridge.
- EMERSON, S. 1948. A physiological basis for some suppressor mutations and possibly for one gene heterosis. *Proc. Nat. Acad. Sci.*, 34, 72-74.
- FISHER, R. A. 1930. *Genetical Theory of Natural Selection*. Oxford Univ. Press, Oxford.
- HARLAND, S. C. 1955. Plant breeding and genetics. *The Advancement of Science*, 44, 383.
- IRWIN, M. R. 1952. *Specificity of Gene Effects in Heterosis*. Iowa State Coll. Press.
- LERNER, I. M. 1954. *Genetic Homeostasis*. Oliver and Boyd, Edinburgh.
- MATHER, K. 1943. Polygenic inheritance and natural selection. *Biol. Rev.*, 18, 32-64.
- MAYR, E. 1942. *Systematics and the Origin of Species*. Columbia Univ. Press.
- SCHMALHAUSEN, J. J. 1949. *Factors of Evolution*. Blakiston, Philadelphia.
- SCHULTZ, F. T., AND BRILES, W. E. 1953. The adaptive value of blood group genes in chickens. *Genetics*, 38, 34-50.
- STERN, C., *et al.* 1952. The viability of heterozygotes for lethals. *Genetics*, 37, 413-449.