Heterozygosity and biomass production in *Salix eriocephala*

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The relationships between heterozygosity at 11 variable isoenzyme loci and total above-ground leafless biomass production were examined in four full-sib families of *Salix eriocephala*, a willow species important in short-rotation intensive-culture feedstock plantations. Relationships were investigated by comparing the performance of heterozygotes with that of corresponding homozygotes in locus-by-locus comparisons, by investigating multiple regression models with individual loci as independent variables, and by employing the adaptive distance model. In all of these analytical approaches, evidence indicated the presence of positive associations between heterozygosity at enzyme loci and biomass production. The theoretical implications of these results are discussed in the specific context of this study.

Keywords: biomass production, fitness, heterozygosity, isoenzymes, *Salix*.

Introduction

The significance of positive associations between heterozygosity and characters related to fitness, such as growth, has been debated for decades in relation to the occurrence of heterosis. Heterosis is the condition where, for a specified quantifiable character, the average score of progeny of two parental stocks is higher than the score of either stock. Because heterosis was originally defined as a property of hybrids, it became closely associated with heterozygosity and synonymous to heterozygote superiority (Zouros & Foltz, 1987). Thus, heterosis has been used as equivalent to overdominance, a term strictly referring to the superiority of the heterozygote for two allelic genes over either corresponding homozygote but not necessarily over any homozygote that may exist in nature. To what extent overdominance is the cause of heterosis remains one of the major unresolved problems in evolutionary and applied genetics (Zouros & Foltz, 1987).

There are two alternative hypotheses accounting for the correlation of heterozygosity with growth and yield: (i) the ‘dominance’ (or ‘inbreeding depression’) hypothesis, stating that the loci under investi-

gation may themselves be selectively neutral and the observed heterozygosity is merely indicative of the degree of genomic heterozygosity which covers deleterious recessives; and (ii) the ‘overdominance’ hypothesis, which states that heterozygosity at the loci examined, or at other loci which are in linkage disequilibrium with the studied loci, leads to increased fitness, i.e. heterozygotes are better *per se* (Smouse, 1986). Heterosis is the inverse of inbreeding depression and can be the result of either increased heterozygosity at a large number of overdominant loci, or decreased expression of a small number of deleterious recessive alleles (Mitton & Grant, 1984).

There is a large amount of published work on the heterozygosity–growth associations concerning annual and perennial crop plants, forest trees, birds, fishes, molluscs, reptiles, and vertebrates including humans (Mitton & Grant, 1984; Zouros & Foltz, 1987; Mitton, 1989). In spite of the diversity of such studies it has been frequently observed that individual alloenzyme heterozygosity is correlated with various quantitative traits, including a number of fitness components and life history characteristics, even though this phenomenon cannot be characterized as general (Zouros, 1987).

This study investigates the associations among isoenzyme heterozygosity and biomass production in *Salix eriocephala* Michx. (section *Cordatae*, subgenus

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Vetrix). *Salix eriocephala* belongs to a group of forest trees that are long-lived, widespread, outcrossing species with high reproductive output, extensive gene flow and large population sizes; they maintain substantial genetic variation within populations, high individual heterozygosity and small chances of linkage disequilibrium associations. They are therefore good candidates for heterozygosity–growth studies. In addition, there is an economic interest in *S. eriocephala*, because it is one of the most promising species in short-rotation intensive-culture biomass plantations for the production of energy, chemicals and fibre in marginal agricultural lands (Aravanopoulos, 1992). Significant quantitative variation has also been detected in this species, indicating its potential for substantial improvement through selection and breeding (Mosseler *et al.*, 1988).

Materials and methods

This study employed the full-sib progenies of a 2×2 balanced factorial mating design, involving female clones E16 and E269, and male clones E263 and E292. The progenies were included in a test plot, established as a randomized complete-block design with each full-sib family represented by a single, four-tree plot in each of six blocks surrounded by a triple border row, at the Ontario Tree Improvement and Forest Biomass Institute (OTIFBI) at Maple, Ontario (44°N, 80°W). Therefore the sample size was 24 trees per family, or 96 trees in total. Heterozygosity was estimated from 11 segregating loci in 11 enzyme systems: acid phosphatase (EC 3.1.3.2, *Acp-2*), aconitase (EC 4.2.1.3, *Aco-2*), alcohol dehydrogenase (EC 1.1.1.1, *Adh-2*), alkaline phosphatase (EC 3.1.3.1, *Alp-1*), cytochrome oxidase (EC 1.9.3.1, *Cto-1*), isocitrate dehydrogenase (EC 1.1.1.42, *Idh-2*), peroxidase (EC 1.11.1.7, *Per-1*), 6-phosphogluconate dehydrogenase (EC 1.1.1.44, *Pgd-1*), phosphoglucosomerase (EC 5.3.1.9, *Pgi-2*), polyphenol oxidase (EC 1.14.18.1, *Ppo-1*) and shikimate dehydrogenase (EC 1.1.1.25, *Sdh-2*). All these loci showed Mendelian inheritance and codominant allelic expression (Aravanopoulos *et al.*, 1994). None of these loci was found to form closely linked groups (Aravanopoulos, 1992).

Seedlings were harvested at $2\frac{1}{2}$ years of age (Mosseler *et al.*, 1988) to measure above-ground leafless oven-dry biomass. This is a typical rotation period for a fast growing species used in short-rotation intensive-culture plantations. Each individual was cut at ground level and was weighed to the nearest 0.01 kg on an electronic balance. Three of the dominant upright stems were randomly selected from

each plant and a 20 cm-long stem section was removed from the stem balance point for determining the moisture content of the wood. Each 20 cm stem section was weighed to the nearest 0.001 g, dried at $105^{\circ}\text{C} \pm 5^{\circ}\text{C}$ for 24 h and then reweighed. The average moisture content of each full-sib family (ratio of oven-dry over green weight of stem sections) was then applied to the green weight of each plant to obtain the oven-dry biomass weight.

Analysis was conducted using SAS (SAS Institute, 1985). To normalize the data and to eliminate as much environmental variance and block effects as possible, biomass measurements and associated plot means were log-transformed and all analyses were based on deviations of each tree from its plot mean, which is also a measure of relative-to-plot growth (Strauss, 1986). The advantage of this experimental layout is that the uniform habitat and the randomized design are expected to contribute more accurate measures for biomass, while problems of environmental effects are minimized (Bush *et al.*, 1987).

The null hypothesis of no associations between heterozygosity and biomass production was tested in three different ways: (i) by comparing performance of heterozygotes with that of corresponding homozygotes in locus-by-locus comparisons (Ledig *et al.*, 1983; Mallet *et al.*, 1986; Wolff & Haack, 1990); (ii) by investigating multiple regression models with individual loci as independent variables (Strauss, 1986; Strauss & Libby, 1987); and (iii) by employing the adaptive distance model (Mitton, 1989; Bush & Smouse, 1991).

In the first approach, for every locus, the mean biomass of all homozygotes was subtracted from the mean biomass of all heterozygotes. Under the null hypothesis of no association between single-locus heterozygosity and biomass, the generation of genotypic groups would be a random process with respect to biomass production. The significance of differences in biomass production between homozygote and heterozygote individuals was examined using *t*-test statistics (Sokal & Rohlf, 1981). The observed ratios of positive to negative differences were tested by χ^2 -tests against the expected 1:1 ratio under the null hypothesis of no association.

In the second approach, heterozygosity at each locus was encoded as an indicator variable: each locus was defined as either 0 (heterozygous condition) or 1 (homozygous condition). Evaluation of the multiple regression models with the genotypic conditions as dependent variables was conducted by *F*-ratio tests and the coefficient of determination (R^2) to assess the proportion of the variation in

Table 1 Allele frequencies at each of the 11 loci for each of the four full-sib families of *Salix eriocephala*

Family	Allele	<i>Aco-2</i>	<i>Acp-2</i>	<i>Adh-2</i>	<i>Alp-1</i>	<i>Cto-1</i>	<i>Idh-2</i>	<i>Per-1</i>	<i>Pgd-1</i>	<i>Pgi-2</i>	<i>Ppo-1</i>	<i>Sdh-2</i>
E269 × E292	<i>A</i>	1.000	0.783	0.500	0.761	1.000	0.370	0.783	0.565	0.739	0.761	0.348
	<i>B</i>	0.000	0.217	0.500	0.239	0.000	0.630	0.217	0.435	0.261	0.239	0.652
E16 × E263	<i>A</i>	0.750	0.500	0.312	1.000	0.771	0.188	0.727	0.667	1.000	0.708	0.208
	<i>B</i>	0.250	0.500	0.688	0.000	0.229	0.812	0.273	0.333	0.000	0.292	0.792
E269 × E263	<i>A</i>	0.771	0.500	1.000	1.000	0.688	0.271	0.717	0.521	1.000	0.818	0.458
	<i>B</i>	0.229	0.500	0.000	0.000	0.312	0.729	0.283	0.479	0.000	0.182	0.542
E16 × E292	<i>A</i>	1.000	0.875	0.792	0.812	1.000	0.250	0.729	0.729	0.792	0.783	0.229
	<i>B</i>	0.000	0.125	0.208	0.188	0.000	0.750	0.271	0.271	0.208	0.217	0.771

biomass production that could be explained by the model (Sokal & Rohlf, 1981). Heterozygote superiority is expected to give a regression line with a negative slope. However, because the multiple regression's response function is multidimensional, partial regression coefficients were examined. The response function was judged to have a 'positive' or 'negative' slope when these partial regression coefficients were predominantly positive or negative for a family (Bush *et al.*, 1987). The relative importance and contribution of a locus to growth was also investigated by ranking the order of inclusion of each locus in a stepwise maximum R^2 improvement regression model within and among families. Significance of each locus contribution was determined by the *F*-tests for each model (Sokal & Rohlf, 1981; SAS Institute, 1985). Average ranks based on the results of the individual families were calculated. Pooled data were also studied by examining the partial sum of squares (Type III SS) of the multiple regressions. The Type III SS indicate the effects of an independent variable separately, holding the effects of the other variables constant, and provide for a given locus a measure of the degree to which heterozygosity at that locus is correlated with biomass production (Sokal & Rohlf, 1981; Koehn *et al.*, 1988). Differences in the rankings between average and pooled data were investigated using the Spearman rank correlation coefficient (Sokal & Rohlf, 1981).

The third approach makes use of a theoretical development, the adaptive distance model (Smouse, 1986). According to this model, if selection is acting upon genotypes to produce heterozygote superiority or overdominance, then the fitness of those genotypes can be predicted by their allelic frequencies. Total above-ground leafless biomass, the ultimate expression of net growth, was regarded as a first approximation of a fitness surrogate for model purposes. Adaptive distances were calculated from genotype frequencies according to Smouse (1986)

and the relationship between log (biomass) and adaptive distances was inferred from multiple regressions. The adaptive distance treatment may provide added information in the case of a locus where at least three genotypes are observed (i.e. *AA*, *Aa*, *aa*), compared to the usage of the binary scoring system which treats both homozygotes in the same way (i.e. *AA* and *aa* = 1; *Aa* = 0). This analysis has been applied in a number of studies on heterozygosity–growth relations, for example in *Pinus rigida* (Bush *et al.*, 1987), *Picea engelmannii*, *Abies lasiocarpa* (Shea, 1989), *Lolium multiflorum* (Mitton, 1989) and *Pinus taeda* (Bush & Smouse, 1991). Model evaluation, investigation of heterozygote superiority and contributions of individual loci were tested as in the second approach.

Results

The allelic frequencies of the 11 variable loci in the four full-sib families studied are presented in Table 1. The descriptive statistics of the total above-ground biomass production of the same families are given in Table 2. The results of the three different experimental approaches used to study heterozygosity–biomass relations are presented below. For the first approach, the results of the comparisons between biomass growth of heterozygotes and that of corresponding homozygotes, in locus-by-locus comparisons, are presented in Table 3. There were

Table 2 Descriptive statistics of biomass production (kg) in *Salix eriocephala*

Family	Mean	Standard deviation	CV	Range
E269 × E263	0.942	0.253	26.86	0.58–1.50
E16 × E263	1.455	0.323	22.20	0.68–1.96
E269 × E292	1.497	0.635	42.42	0.16–3.26
E16 × E292	1.130	0.433	38.32	0.41–2.24

Table 3 Effects of individual loci in *Salix eriocephala* given as δ = (mean biomass of heterozygotes) – (mean biomass of homozygotes) for each family and enzyme

Locus	E269 × E292	E16 × E263	E269 × E263	E16 × E292	$\delta > 0$	$\delta < 0$	Pooled
<i>Aco-2</i>	LNS	0.001	0.514***	LNS	2	0	0.515
<i>Acp-2</i>	0.015	LNS	LNS	0.133***	2	0	0.148
<i>Adh-2</i>	LNS	0.045***	LNS	0.052*	2	0	0.057
<i>Alp-1</i>	–0.059	LNS	LNS	0.007	1	1	–0.052
<i>Cto-1</i>	LNS	–0.083**	0.011	LNS	1	1	–0.072
<i>Idh-2</i>	0.028	0.004	0.086	–0.017	3	1	0.101
<i>Per-1</i>	–0.194***	0.114***	–0.022	0.046*	2	2	–0.056
<i>Pgd-1</i>	–0.010	0.077**	LNS	–0.007	1	2	0.060
<i>Pgi-2</i>	0.046	LNS	LNS	0.001	2	0	0.047
<i>Ppo-1</i>	0.170***	0.025	0.158**	–0.096*	3	1	0.257
<i>Sdh-2</i>	–0.034	0.105***	0.233***	0.127***	3	1	0.431
Sign. $\delta > 0$	1/2	4/5	3/3	4/5	12	3	
<i>P</i>					<0.05		

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ (*t*-tests).

δ -values are in ‘antilog’ scale; LNS, locus not segregating in that family.

31 possible comparisons. The total ratio of significant positive:negative differences for every locus and family was significantly different from 1:1.

For the second and third approaches, the results of the multiple regression models investigated are shown in Table 4. Ten models were tested. Results of the separate family models were coincident. There was no marked difference between the results obtained from binary scores and those obtained from adaptive distances, in terms of *F*-ratios and average R^2 values across families. The latter were not significantly different between the two types of analysis ($t_6 = 1.32$). A noteworthy result for both types of analysis was that the pooled data produced models that could not effectively account for any of the variability in biomass production. The pooled data model accounted for 3.6% of the biomass varia-

tion, whereas individual families on average accounted for 29% (binary scores; Table 4). Subsets of the loci employed in the stepwise regression procedure for determining locus ranks resulted in significant associations between biomass productivity in one family, E16 × E263 (Table 5).

The partial regression coefficients of the above models that were used to assess heterozygote superiority are presented in Table 6. In individual families and in the pooled data, most of the partial regression coefficients were negative; therefore the multiple regression response function was also considered to be negative. The signs and magnitudes of loci on the other hand were not constant from family to family. This was observed in both the binary scores and adaptive distances analytical approaches. The overall split of positive and nega-

Table 4 Regression results in *Salix eriocephala*; multilocus effects. Coefficient of determination and *P* (associated with *F*-ratio) for the regression models of log (biomass) on binary scores and adaptive distances (SD in parentheses)

Family	Binary scores		Adaptive distances	
	R^2	<i>P</i>	R^2	<i>P</i>
E269 × E292	0.327	0.578	0.311	0.620
E16 × E263	0.431	0.266	0.423	0.373
E269 × E263	0.136	0.837	0.205	0.724
E16 × E292	0.265	0.807	0.265	0.807
Average	0.290 (0.123)		0.301 (0.092)	
Pooled	0.036	0.987	0.071	0.846

Table 5 Rankings of the contribution of each locus to biomass production in *Salix eriocephala* based on the order of inclusion in the regression models of individual families and pooled data. Average ranks of the family data are also presented

Rank	Pooled	E269 × E292	E16 × E263	E269 × E263	E16 × E292	Average rank
1	<i>Per-1</i>	<i>Per-1</i>	<i>Sdh-2</i>	<i>Sdh-2</i>	<i>Sdh-2</i>	<i>Sdh-2</i> = 1.25
2	<i>Aco-2</i>	<i>Sdh-2</i>	<i>Pgd-1</i> *	<i>Ppo-1</i>	<i>Acp-2</i>	<i>Per-1</i> = 3.25
3	<i>Adh-2</i>	<i>Pgd-1</i>	<i>Per-1</i> *	<i>Idh-2</i>	<i>Alp-1</i>	<i>Alp-1</i> = 4.00
4	<i>Pgi-2</i>	<i>Ppo-1</i>	<i>Idh-2</i>	<i>Aco-2</i>	<i>Per-1</i>	<i>Ppo-1</i> = 4.25
5	<i>Ppo-1</i>	<i>Alp-1</i>	<i>Cto-1</i>	<i>Per-1</i>	<i>Ppo-1</i>	<i>Pgd-1</i> = 4.33
6	<i>Cto-1</i>	<i>Pgi-2</i>	<i>Ppo-1</i>	<i>Cto-1</i>	<i>Pgi-2</i>	<i>Acp-2</i> = 4.50
7	<i>Alp-1</i>	<i>Acp-2</i>	<i>Aco-2</i>		<i>Adh-2</i>	<i>Cto-1</i> = 5.50
8	<i>Idh-2</i>	<i>Idh-2</i>	<i>Adh-2</i>		<i>Pgd-1</i>	<i>Aco-2</i> = 5.50
9	<i>Pgd-1</i>				<i>Idh-2</i>	<i>Idh-2</i> = 6.00
10	<i>Acp-2</i>					<i>Pgi-2</i> = 6.00
11	<i>Sdh-2</i>					<i>Adh-2</i> = 7.50

* $P < 0.05$ (F -ratio test).

tive partial regression coefficients in the four families was significantly different from a 1:1 ratio expected with no association between biomass and heterozygosity (Table 6, $\chi^2_1 = 3.903$, $0.025 < P < 0.050$).

The relative contributions of individual loci to the overall relationship between heterozygosity and biomass, within and among families (Table 5), indicated that different loci emerged as more important in different families with the exception of *Sdh-2*, which ranked first in three families. When family data were pooled, the ranking of loci was different from that of individual families as well as from their average ranks, and no significant correlation existed

(Spearman rank correlation coefficient: $r_s = 0.975$, $0.30 < P < 0.40$; Fig. 1).

Discussion

The results of biomass differences in locus-by-locus comparisons showed that in most cases individuals heterozygous at one locus produced more biomass than individuals homozygous for that locus. Significant deviations from 1:1 were found in the numbers of the 'positive' and 'negative' statistically significant differences. The ratio showed that the number of cases where heterozygotes produced more biomass was significantly higher than the number of cases

Table 6 Partial regression coefficients of multiple regression models in *Salix eriocephala* (binary scores used; LNS, locus not segregating in that family)

Family	E269 × E292	E16 × E263	E269 × E263	E16 × E292	Total	Pooled
<i>Aco-2</i>	LNS	−0.005	−0.047	LNS		−0.004
<i>Acp-2</i>	−0.004	LNS	LNS	−0.067		−0.026
<i>Adh-2</i>	LNS	−0.007	LNS	0.016		−0.002
<i>Alp-1</i>	0.071	LNS	LNS	0.036		0.019
<i>Cto-1</i>	LNS	0.029	−0.048	LNS		0.022
<i>Idh-2</i>	−0.036	−0.022	−0.042	−0.008		−0.015
<i>Per-1</i>	0.104	−0.036	−0.016	−0.016		0.029
<i>Pgd-1</i>	−0.037	−0.033	LNS	0.010		−0.012
<i>Pgi-2</i>	0.037	LNS	LNS	−0.019		−0.013
<i>Ppo-1</i>	−0.023	−0.024	−0.035	0.038		−0.004
<i>Sdh-2</i>	0.003	0.048	−0.088	−0.055		−0.021
(+) signs	4	2	0	4	10	3
(−) signs	4	6	6	5	21	8

Fig. 1 Variation in the order of importance of individual loci to the overall relationship of heterozygosity and biomass in *Salix eriocephala*; lines connect ranks of each locus.

where heterozygotes produced less biomass than corresponding homozygotes. The results also suggested that the performance of one-locus heterozygotes may vary across families. In general, the number of loci for which heterozygotes grew better than homozygotes was high, but heterozygotes at the same locus did not do so in every family. The magnitude of the biomass differences varied among enzyme loci and families.

The results using regression of biomass on binary scores and adaptive distances were consistent. The amount of biomass variation accounted for by these approaches was virtually the same. As noted, the use of the adaptive distances method was proposed to investigate if the differential treatment of homozygotes in a model will result in a better explanation of the variability in biomass. The adaptive distances approach did not produce better models. This technique may be insensitive to mating schemes other than random, especially when only two alleles are present at a locus. In particular, adding information about differences in relative allele frequencies could not be accomplished when the progeny for a single locus segregated in a 1:1 fashion. In these cases adaptive distances and binary scores have identical performance. The technique provides added information in the other types of genotypic segregation in progenies, which occurred for about 10% of the loci on average over different families. Clearly, differential treatment of loci at that level did not result in regression models that explained a higher amount of

the variation observed in biomass compared with the use of binary scores. Accordingly, the rest of the discussion will focus on the binary scores approach.

The interpretation of the regression results indicated that heterozygosity–biomass associations were less pronounced from this type of analysis, than from the analysis of separate loci. When all loci were used, a significant correlation was observed in only one family. The reduced efficiency of the pooled data models demonstrated that trends in heterozygosity–biomass relations are not the same across families; pooling data may mask associations that could exist within families. Heterozygote superiority in *S. eriocephala* was indicated by the response functions of the multiple regressions which were predominantly negative.

Rankings indicated that the importance of loci in different families varied; *Sdh-2* was an exception. Both the proportional contribution of loci and the sign of the association varied and may have contributed to the diversity observed. The same trends were also observed in the single-locus analysis. The absence of association between the rankings when ranks were averaged across families and when ranks were obtained by pooling individual family data, further supported the suggestion of family-specific heterozygosity–growth relationships.

In summary, the analysis of heterozygosity–biomass relations revealed the following: (i) there was a positive association between isoenzyme heterozygosity and biomass in *S. eriocephala* and this

was indicated by both single-locus and multilocus analyses, and (ii) heterozygosity–growth associations appeared to be family-specific for most loci.

The existence of associations in *S. eriocephala* allows some reference to the potential underlying mechanisms that may account for heterozygosity–growth relationships. If the dominance hypothesis is correct, then it is expected that full-sib families should not show any internal associations between heterozygosity and a surrogate of fitness, because full-sibs have the same inbreeding coefficient. In *S. eriocephala*, associations within full-sib families therefore cannot be explained by differences in degree of relationship among individuals. Similar results for full-sibs have been reported by Pierce & Mitton (1982) and Leary *et al.* (1987). If an overdominant explanation for the observed associations is considered, it is still very difficult to infer whether associations in full-sib families result from the enzyme loci themselves (true overdominance hypothesis), or from closely linked loci (associative overdominance hypothesis). Full-sibs heterozygous at isoenzyme loci are expected to be more heterozygous at the chromosomal segments marked by those loci (Leary *et al.*, 1987). On the other hand, Zouros (1987) has argued that sporadic and inconsistent patterns of genotypic effects on growth in full-sib families could be expected from associative overdominance, but are not consistent with the direct involvement of enzyme loci. This study revealed inconsistent patterns across families and loci. The evidence is limited, nevertheless it could be postulated that the results are closer to the expectations of the associative overdominance hypothesis than to those of the true overdominance hypothesis. Similar results of inconsistent locus performance in full-sibs were also detected in studies of marine molluscs (Beaumont *et al.*, 1983; Gaffney & Stott, 1984; Mallet *et al.*, 1986). An exception to the above pattern was the high ranking of *Sdh-2* in every family. Shikimate dehydrogenase participates in the synthesis of aromatic metabolites. An indirect SDH involvement with growth may be related to increased lignin production, possible effects of increased disease resistance (through increased facilitation of phenolic compound production by specific SDH genotypes), or alternatively SDH may be tightly linked to a major gene that affects growth.

The positive associations of heterozygosity and biomass in *S. eriocephala* may have implications in breeding if verified by further independent studies. Under the overdominance explanation of the observed associations, basic selection theory dictates that most loci in a population will be polymorphic

and consequently a typical individual would be expected to be heterozygous for a large fraction of its genome. There can be no best genotype that can breed ‘true’, and the largest possible gains in a breeding experiment will occur by developing inbred lines and then cross-fertilizing (Singh & Zouros, 1980; Zouros & Foltz, 1987). Although population or progeny selection can be assisted by enzyme markers, both are expected to generate lower gains than the hybridization of inbred lines could accomplish.

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References

- ARAVANOPOULOS, F. A. 1992. *Dynamics of Electrophoretic Spectra in Families of Salix eriocephala Michx. and S. exigua Nutt. and their Implementation in Willow Breeding Research*. Ph.D. Thesis, University of Toronto.
- ARAVANOPOULOS, F. A., ZSUFFA, L. AND CHONG, K. X. 1994. Inheritance analysis of isoenzyme genes in *Salix eriocephala* Michx. *J. Hered.*, **85**, 381–388.
- BEAUMONT, A. R., BEVERIDGE, C. M. AND BUDD, M. D. 1983. Selection and heterozygosity within single families of the mussel *Mytilus edulis* (L.). *Mar. Biol. Lett.*, **4**, 151–161.
- BUSH, R. M. AND SMOUSE, P. E. 1991. The impact of electrophoretic genotype on life history traits in *Pinus taeda*. *Evolution*, **45**, 481–498.
- BUSH, R. M., SMOUSE, P. E. AND LEDIG, F. T. 1987. The fitness consequences of multiple locus heterozygosity: the relationship between heterozygosity and growth rate in pitch pine (*Pinus rigida* Mill.). *Evolution*, **41**, 787–798.
- GAFFNEY, P. M. AND STOTT, T. M. 1984. Genetic heterozygosity and production traits in natural and hatching populations of bivalves. *Aquaculture*, **42**, 289–302.

- KOEHN, R. K., DIEHL, W. J. AND STOTT, T. M. 1988. The differential contribution by individual enzymes of glycolysis and protein catabolism to the relationship between heterozygosity and growth rate in the coot clam *Mulinia lateralis*. *Genetics*, **118**, 121–130.
- LEARY, R. B., ALLENDORF, F. W. AND KNUDSEN, K. L. 1987. Differences in inbreeding coefficients do not explain the association between heterozygosity at allozyme loci and developmental stability in rainbow trout. *Evolution*, **41**, 1413–1415.
- LEDIG, F. T., GURIES, R. P. AND BONEFELD, B. A. 1983. The relation of growth to heterozygosity in pitch pine. *Evolution*, **37**, 1227–1238.
- MALLET, A. L., ZOUROS, E., GARTNER-KEPAY, K. E. AND FREEMAN, K. R. 1986. Genetics of growth in blue mussels: family and enzyme heterozygosity effects. *Mar. Biol.*, **92**, 475–482.
- MITTON, J. B. 1989. Physiological and demographic variation associated with allozyme variation. In: Soltis, D. E. and Soltis, P. S. (eds) *Isozymes in Plant Biology*, pp. 127–145. Dioscorides Press, Portland, OR.
- MITTON, J. B. AND GRANT, M. C. 1984. Associations among protein heterozygosity, growth rate and developmental homeostasis. *Ann. Rev. Ecol. Syst.*, **15**, 479–499.
- MOSSELER, A., ZSUFFA, L., STOEHR, M. U. AND KENNEY, W. A. 1988. Variation in biomass production, moisture content and specific gravity in some North American willows. *Can. J. Forest Res.*, **18**, 1535–1540.
- PIERCE, B. A. AND MITTON, J. B. 1982. Allozyme heterozygosity and growth in tiger salamander *Ambystoma tigrinum*. *J. Hered.*, **73**, 250–253.
- SAS INSTITUTE 1985. *SAS User's Guide: Statistics*. SAS Institute Publ., Cary, NC.
- SHEA, K. L. 1989. The relationship between heterozygosity and fitness in Engelmann spruce and subalpine fir. *Am. J. Bot.*, **76** (Suppl.), 153–154.
- SINGH, S. M. AND ZOUROS, E. 1980. Genetics of growth rate in oysters and its implications for aquaculture. *Can. J. Genet. Cytol.*, **23**, 119–130.
- SMOUSE, P. E. 1986. The fitness consequences of multiple locus heterozygosity under the multiplicative overdominance and inbreeding depression models. *Evolution*, **40**, 956–967.
- SOKAL, R. R. AND ROHLF, F. J. 1981. *Biometry*, 2nd edn. W.H. Freeman and Co., New York.
- STRAUSS, S. H. 1986. Heterosis at allozyme loci under inbreeding and crossbreeding in *Pinus attenuata*. *Genetics*, **113**, 115–134.
- STRAUSS, S. H. AND LIBBY, W. J. 1987. Allozyme heterosis in radiata pine is poorly explained by overdominance. *Am. Nat.*, **130**, 879–890.
- WOLFF, K. AND HAECK, J. 1990. Genetic analysis of ecologically relevant morphological variability in *Plantago lanceolata*. VI. The relation between allozyme heterozygosity and some fitness components. *J. Evol. Biol.*, **3**, 243–255.
- ZOUROS, E. 1987. On the relation between heterozygosity and heterosis: an evaluation of the evidence from marine molluscs. *Isozymes*, **15**, 255–270.
- ZOUROS, E. AND FOLTZ, D. W. 1987. The use of allelic isozyme variation for the study of heterosis. *Isozymes*, **13**, 1–59.