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Partitioning of population genetic variance under multiplicative-epistatic gene action

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Abstract Determining the way in which different QTLs interact (epistasis) in their effects on the phenotype is crucial to many areas in population genetics and evolutionary biology. For example, in the founder event, a separated population readapts to a new environment through the release of cryptic gene-gene interactions. In hybrid zones, hybrid speciation must be subjected to natural selection for epistasis resulting from genomic recombinations between different species. However, there is a severe shortage of relevant methodologies to estimate epistatic genetic effects and variances. A statistical model has recently been proposed to estimate the number of QTLs, their genetic effects and allelic frequencies in segregating populations. This model is based on multiplicative gene action and derived from a two-level intra- and interspecific mating design. In this paper, we formulate a statistical procedure for partitioning the genetic variance into additive, dominant and various kinds of epistatic components in an intra- or mixed intra- and interspecific hybrid population. The procedure can be used to study the genetic architecture of fragmented populations and hybrid zones, thus allowing for a better recognition of the role of epistasis in evolution and hybrid speciation. A real example for two *Populus* species, *P. tremuloides* and *P. tremula*, is provided to illustrate the procedure. In this example, we found that considerable new genetic variation is formed through genomic recombination between two aspen species.

Key words Additive genetic variance · Aspen · Dominant genetic variance · Epistatic genetic variance · Founder effect · Hybrid speciation · Multiplicative epistasis · Outcrossing species

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Introduction

Epistasis has been suggested to play a critical role in affecting two major evolutinary processes of speciation, founder effects (Giddings et al. 1989) and hybridization (Harrison 1993). When a population of small size becomes separated from a larger parental population, the founding event will be effective to produce a new genetic environment that leads the separated population to better adapt to the population bottleneck (Templeton 1979, 1980; Gavrilets and Hastings 1996). This phenomenon in which physiologically interacting genes readapt to one another in new genetic alignments is called the genetic "revolution" by Mayr (1954). In the wild, much quantitative variation that appears as a small effect may virtually hide large unexpressed molecular effects (Kacser and Burns 1981; Keightley and Kacser 1987). Once genotypic frequencies in the populations are disturbed by selection, or population bottleneck, such cryptic molecular variation can act as a potential source of strong phenotypic effects via gene-gene interactions (Carson and Templeton 1984; Goodnight 1987, 1988, 1995; Tachida and Cockerham 1989; Whitlock et al. 1995). In a number of evolutionary quantitative genetic studies (mainly on *Drosophila*), for example, an increase in additive genetic variance was noted in populations passing through bottlenecks (Bryant et al. 1986; Lopez-Fanjul and Villaverde 1989; Shearn 1989; Bryant and Meffert 1990, 1993, 1995, 1996; Goodnight 1995). However, Cheverud and Routman (1996) recently showed that some forms of epistasis could also result in a temporary reduction in additive genetic variance during a population bottleneck.

As a most important source of genetic variation that is required for major evolutionary advances, interspecific hybridization is suggested to be another important evolutionary force of species formation (Stebbins 1959). There has been much experimental evidence to show that natural hybrids can serve as founding populations for new species (Arnold et al. 1991; Rieseberg et al. 1995). Interactions between divergent species' genomes are of-

ten viewed as uniformly disharmonious (Mayr 1963), resulting in hybrid inviability or sterility. However, the successful origin of new diploid species by means of hybridization raises the possibility that interactions between parental species' genes are not universally unfavorable (Rieseberg 1995; Rieseberg et al. 1995). For example, Rieseberg et al. (1996) suggested, from molecular data in crosses between two wild sunflower species, *Helianthus annuus* and *H. petiolaris*, that although the majority of interspecific gene interactions were indeed unfavorable or neutral, a small percentage of alien genes did appear to interact favorably in hybrids. A similar conclusion was also reached for crosses between two species of Louisiana iris, *Iris fulva* and *I. Brevicaulis* (Burke et al. 1998). In these experiments, the authors suggested that favorable gene interactions provide the raw material for adaptive evolution in hybrid taxa.

Although epistasis may influence the formation of species, it is very difficult to quantify its contribution to the genetic variance in a population. Cheverud and Routman (1995) proposed a method based on molecular markers to specify the contribution of epistasis to additive, dominant and interaction genetic variances. Several techniques were developed to test for epistasis between deleterious mutations (de Visser et al. 1996, 1997a; West et al. 1998). However, these methods cannot be used to study the role of gene interactions between individual quantitative trait loci (QTLs) in the evolutionary process of hybridization because the genetic composition and structure of a hybrid population are not considered. By using a two-level intra- and interspecific mating design, Wu and Li (1999) developed a novel analytical method for estimating epistatic parameters, such as the number of QTLs and their gene effects and allelic frequencies in a population, that could be important for species differences and hybridization. This method is based on a genetic inference that a complex trait is affected by multiplicative action in pairs from a suite of QTLs, as has been demonstrated both theoretically and experimentally (Arunachalam 1977; Minvielle 1987; Griffing 1990; Schnell and Cockerham 1992; Zhang et al. 1994; Li and Wu 1996).

The primary objective of this paper is to formulate a procedure for estimating the contribution of multiplicative epistasis to genetic variance components in an intra- or mixed intra- and interspecific hybrid population, followed by an example derived from crosses between two aspen species, *Populus tremuloides* and *P. tremula*. *Populus tremuloides*, native to North America, has the name of quaking aspen for the distinctive fluttering of its leaves, even in the most gentle breezes. Early French-Canadian trappers called the tree an aspen because of its similarity to *P. tremula*, a closely related species in Europe and Asia. Interspecific hybridization between these two aspen species has been attractive to tree breeders for almost a century because of strong heterosis for growth and productivity in their hybrid progeny (Heimburger 1936; Einspahr 1984; Li and Wu 1996). By means of Wu and Li's (1999) statistical method based on a two-locus multiplicative epistasis model, a number of genetic parameters, including gene effects at individual QTLs and their allelic frequencies in the populations, were estimated from an intra- and interspecific mating design of *P. tremuloides* and *P. tremula*. These parameters are further employed to demonstrate the statistical procedures for partitioning genetic variances into additive, dominant, and additive×additive, additive×dominant, dominant×additive and dominant×dominant epistatic components in a pure intraspecific or a mixed intra- and interspecific hybrid population, respectively.

Multiplicative epistasis and its estimation

Consider two outcrossing species, E and E', with different allelic systems. In each species, dialleles are assumed, with A (dominant) and a (recessive) for E and A' (dominant) and a' (recessive) for E' at a QTL. Dialleles at another epistatically related QTL are denoted by B, b, B' and b', respectively. Allelic frequencies at these two QTLs in each population are defined as

Population	E				E'			
Allele	A	a	B	b	A'	a'	B'	b'
Frquency	p_1	q_1	p_2	q_2	p'_1	q'_1	p'_2	q'_2

In species E's population, the values of three genotypes, dominant homozygote, heterozygote and recessive homozygote, are defined as a_{11}, a_{12}, and a_{22} at QTL A/a, and b_{11}, b_{12}, and b_{22} at QTL B/b. The corresponding genotypic values in the population of species E' are denoted a'_{11}, a'_{12} and a'_{22}, and b'_{11}, b'_{12} and b'_{22}. When the two species are crossed, new heterozygotes, each with one allele from E and the other from E', are formed whose genotypic values are defined as A_{11} for AA', A_{12} for Aa', A'_{12} for $A'a$ and A_{22} for aa' at QTL A/A'. The corresponding genotypic values at QTL B/B' are B_{11}, B_{12}, B'_{12}, and B_{22}, respectively. A multiplicative epistasis model assumes that genotypes at a pair of QTLs have genotypic values equal to the product of genotypic values at the two different QTLs (Minvielle 1987; Charlesworth 1990; Schnell and Cockerham 1992; Otto and Feldman 1997). For example, if genotypic values are a_{11} for AA and b_{11} for BB, the genotypic value of $AABB$ is $a_{11}b_{11}$. The rest may be inferred by analogy.

Wu and Li (1999) proposed a new statistical genetic method to simultaneously estimate the number of QTLs and their genetic values and allelic frequencies in the populations of the two outcrossing species. This method is based on four genetic assumptions, which are (1) all underlying QTLs are independent of each other, i.e. every gene of interest is on a separate chromosome; (2) the distribution of genotypic values among loci is of an exponential kind and may be approximated by a geometric series (see Mackay 1996); (3) variation in gene frequency across loci may follow a Poisson distribution; and (4) the recessive allele at the gene locus of the smallest effect has the largest frequency, gradually decreasing until

the last locus with the largest effect. Following the description of each assumption, Wu and Li (1999) described strong evidence for the assumptions from current quantitative and molecular experiments.

The partitioning of genetic variance

Estimates of genotypic values and allelic frequencies at individual QTLs can be used to estimate the components of genetic variance in both intra- and interspecific hybrid populations. Based on the model for multiplicative interaction between a pair of QTLs, the value of a genotype can be partitioned into the overall mean and genetic effects of various kinds. We will present the genetic variance components for two kinds of populations: pure intraspecific and mixed intra- and interspecific.

Intraspecific hybrid population

In the intraspecific cross $E\times E$, the value of a genotype under the multiplicative epistatic model can be written, for a pair of epistatically related QTLs A/a and B/b, as (Schnell and Cockerham 1992):

$$\begin{aligned} a_{ij}\, b_{kl} = {} & \mu+(r_i+r_j)\mathrm{A}_a+r_i\, r_j\Delta_a+(s_k+s_l)\mathrm{A}_b+s_k\, s_l\Delta_b \\ & +(r_i+r_j)\,(s_k+s_l)\mathrm{AA}_{ab}+(r_i+r_j)s_k\, s_l\mathrm{A}\Delta_{ab} \\ & +r_i\, r_j(s_k+s_l)\Delta\mathrm{A}_{ab}+r_i\, r_j\, s_k\, s_l\Delta\Delta_{ab} \end{aligned}$$

where the subscripts i and j denote the ith allele at QTL A/a (i, j=1, 2), subscripts k and l denote the kth and lth allele at QTL B/b (k, l=1, 2) and subscripts a and b denote QTL A/a and B/b, respectively; $r_1=q_1$, $r_2=-p_1$, $s_1=q_2$, $s_2=-p_2$; μ is the overall mean, A is the additive effect, Δ is the dominance effect and AA, AΔ, ΔA and $\Delta\Delta$ are the additive×additive, additive×dominant, dominant×additive and dominant×dominant effects, respectively, whose values are expressed as:

$$\mu=\mu_a\,\mu_b$$

$$\mu_a=p^2_1\, a_{11}+2p_1\, q_1\, a_{12}+q^2_1\, a_{22}\;\mu_b=p^2_2\, b_{11}+2p_2\, p_2\, b_{12}+q^2_2\, b_{22}$$

$$\mathrm{A}=\alpha_a\,\mu_b\alpha_a=p_1(a_{11}-a_{12})+q_1(a_{12}-a_{22})\;\Delta_a=\delta_a\,\mu_b$$

$$\delta_a=a_{11}-2a_{12}+a_{22}\;A_b=\alpha_b\,\mu_a$$

$$\alpha_b=p_2(b_{11}-b_{12})+q_2(b_{12}-b_{22})\;\Delta_b=\delta_b\,\mu_a$$

$$\delta_b=b_{11}-2b_{12}+b_{22}$$

$$\mathrm{AA}_{ab}=\alpha_a\,\alpha_b$$

$$\mathrm{A}\Delta_{ab}=\alpha_a\,\delta_b$$

$$\Delta\mathrm{A}_{ab}=\delta_a\,\alpha_b$$

$$\Delta\Delta_{ab}=\delta_a\,\delta_b$$

By letting i, j=1, 2 with all possible combinations for genotype a_{ij}, it is not difficult to derive the additive and dominant genetic variances at QTL A/a:

$$V_{A\leftarrow A/a}=2p_1\, q_1\,\mathrm{A}^2_a$$

$$V_{D\leftarrow A/a}=p^2_1\, q^2_1\,\Delta^2_a$$

Similar equations can be easily derived for QTL B/b. The epistatic variance of various kinds (i.e., additive×additive, additive×dominant, dominant×additive and dominant×dominant) due to interactions between the two QTLs are:

$$V_{AA\leftarrow(A/a)(B/b)}=4p_1\, q_1\, p_2\, q_2\,[\mathrm{AA}_{ab}]^2$$

$$V_{AD\leftarrow A/a)(B/b)}=2p_1\,\mathrm{q}_1\; p^2_2\, q^2_2\,[\mathrm{A}\Delta_{ab}]^2$$

$$V_{DA\leftarrow(A/a)(B/b)}=2p^2_1\, q^2_1\;\; p_2\,\mathrm{q}_2\,[\Delta\mathrm{A}_{ab}]^2$$

$$V_{DD\leftarrow(A/a)(B/b)}=p^2_1\, q^2_1\;\; p^2_2\, q^2_2\,[\Delta\Delta_{ab}]^2$$

The same procedures are applicable to the intraspecific cross, $E'\times E'$.

Mixed intra- and interspecific hybrid populations

If species E and E' are mixed with the same number of individuals, their progenies will include those from intra- ($E\times E$ and $E'\times E'$) and interspecific crosses ($E\times E'$ and $E'\times E$). Obviously, in such a mixed progeny population, there are ten different genotypes at a QTL, i.e. AA, Aa, aa, $A'A'$, $A'a'$, $a'a'$, AA', Aa', $A'a$ and aa' at locus A/A', whose allelic frequencies are $p_A=p_1/2$ for A, $p_{A'}=p'_1/2$ for A', $p_a=q_1/2$ for a, and $p_{a'}=q'_1/2$ for a'. The corresponding allelic frequencies for QTL B/B' are $p_B=p_2/2$, $p_{B'}=p'_2/2$, $p_b=q_2/2$, and $p_{b'}=q'_2/2$. For simplicity, it is assumed that recessive alleles have very small effects in both species and, thus, we can set $a_{22}=a'_{22}$. Using the same principle as that for a diallelic case (see above), the genotypic value at a pair of multiplicative QTLs can be decomposed into the overall mean and genetic effects for the mixed progenies (Wu 1999). Based on such decomposition, formulas for estimating additive, dominant and additive×additive×additive×dominant, dominant×additive and dominant×dominant epistatic variances have been derived for a mixed hybrid population of two species (Wu 1999).

If no linkage is assumed among all QTLs of interest, the total additive variance, the total dominance variance and the total epistatic variance of various kinds accounted for by these QTLs are derived based on the expressions of $V_{A\leftarrow(A/A')}$, $V_{D\leftarrow(A/A')}$, $V_{AA\leftarrow(A/A')(B/B')}$, $V_{AD\leftarrow(A/A')(B/B')}$, $V_{DA\leftarrow(A/A')(B/B')}$ and $V_{DD\leftarrow(A/A')(B/B')}$ (Wu 1999). To facilitate such mathematical manipulations, however, two assumptions were made; that is, the distribution of genotypic values across QTLs can be fitted by the geometric series and allelic frequencies follow a Poisson distribution.

An example: population genetic structure in aspens

Li and Wu (1996) used two different aspen species, *Populus tremuloides* and *P. tremula*, to make a two-level factorial mating design including both intra- and interspe-

Table 1 The estimates for the genotypic values[a] and allelic frequencies at the two QTLs of the largest effects on the phenotype for second-year volume growth in the intra- and interspecific crosses of *P. tremuloides* and *P. tremula* (adapted from Wu and Li 1999)

Genotype	Genotypic value at locus *A*/*A*′/*a*	Genotypic value at locus *B*/*B*′/*b*
AA	a_{11}=8.31	b_{11}=5.98
Aa	a_{12}=8.93	b_{12}=5.80
Aa	a_{22}=0*	b_{22}=0*
A′*A*′	a'_{11}=9.20	b'_{11}=8.19
A′*a*′	a'_{12}=7.20	b'_{12}=4.03
a′*a*′	a'_{22}=0*	b'_{22}=0*
AA′	A_{11}=21.23	B_{11}=7.64
Aa′	A_{12}=15.20	B_{12}=5.17
A′*a*	A'_{12}=13.47	B'_{12}=7.81
Aa′	A_{22}=0*	B_{22}=0*
Recessive (spp.)	Recessive frequency at locus *A*/*A*′/*a*	Recessive frequency at locus *B*/*B*′/*b*
A (*E*)	q_1=0.48	q_2=0.35
A′ (*E*′)	q_1'=0.32	q_2'=0.36

[a] An asterisk indicates that the parameter was fixed at this value

cific hybridization. Interspecific crosses between these two species are very easy and often produce remarkable F_1 heterosis (Heimburger 1936; Einspahr 1984). Genetic parameters that underlie species differences in stem volume growth at ages 2 years were estimated using a multiplicative epistatic model developed by Wu and Li (1999). These parameters include the values of three genotypes and allelic frequencies at the reference QTL in either intraspecific hybrid population of *P. tremuloides* and *P. tremula* and the values of the four genotypes formed through interspecific hybrid hybridization. Variation in these parameters across different QTLs was estimated under an assumed geometric series distribution for genotypic values and a Poisson distribution for allelic frequencies. When recessive alleles were restricted to have null effects in both intra- and interspecific hybrid populations, i.e. $a_{22}=a'_{22}=A_{22}=0$, the data seemed to be well fit by the multiplicative-epistatic model (χ^2=0.428 with *df*, *P*=0.80). Parameter estimations under this restriction were obtained for the two QTLs of the largest effects on the phenotypes (Table 1).

Using the procedure developed in this paper, we estimate the additive, dominant and epistatic variance components of various kinds contributed by the two largest QTLs in each intraspecific hybrid population and a mixed intra- and interspecific hybrid population. For clarity, we present the steps for calculating variance components due to these two QTLs in the *E*×*E* intraspecific hybrid population. The corresponding variance components for the mixed population were calculated using the formulas given in Wu (1999).

Mean values at the two QTLs, *A*/*a* and *B*/*b*, in the intraspecific hybrid population of *E*×*E*:

$$\mu_a=p^2_1\,a_{11}+2p_1\,q_1\,a_{12}+q^2_1\,a_{22}$$
$$=(1-0.48)\times8.31+2\times(1-0.48)\times0.48\times8.93+0.48\times0=6.71$$

$$\mu_b=p^2_2\,b_{11}+2p_2\,q_2\,b_{12}+q^2_2\,b_{22}$$
$$=(1-0.35)\times5.98+2\times(1-0.35)\times0.35\times5.90+0.35\times0=5.16$$

Additive deviations at the two QTLs:

$$\alpha_a=p_1(a_{11}-a_{12})+q_1\,(a_{12}-a_{22})=(1-0.48)\times(8.31-8.93)$$
$$+0.48\times(8.93-0)=3.96$$

$$\alpha_b=p_2\,(b_{11}-b_{12})+q_2\,(b_{12}-b_{22})=(1-0.35)\times(5.98-5.80)$$
$$+0.35\times(58.0-0)=2.16$$

Dominant deviations at the two QTLs:

$$\delta_a=a_{11}-2a_{12}+a_{22}=8.31-2\times8.93+0=-9.55$$

$$\delta_b=b_{11}-2b_{12}+b_{22}=5.98-2\times5.80+0=-5.63$$

Additive effects at the two QTLs:

$$A_a=\alpha_a\,\mu_b=3.96\times5.16=20.43$$

$$A_b=\alpha_b\,\mu_a=6.71\times2.17=14.58$$

Dominant effects at the two QTLs:

$$\Delta_a=\delta_a\,\mu_b=-9.55\times5.16=-49.28$$

$$\Delta_b=\delta_b\,\mu_a=-5.63\times6.70=-37.72$$

Epistatic effects between the two QTLs:

$$AA_{ab}=\alpha_a\,\alpha_b=3.96\times2.16=8.55$$

$$A\Delta_{ab}=\alpha_a\,\delta_b=3.96\times(-5.63)=-22.29$$

$$\Delta A_{ab}=\delta_a\,\alpha_b=-9.55\times2.16=-20.63$$

$$\Delta\Delta_{ab}=\delta_a\,\delta_b=-9.55\times(-5.63)=53.77$$

Additive genetic variances at the two QTLs:

$$V_{A\leftarrow A/a}=2p_1\,q_1\,A^2_a=2\times(1-0.48)\times0.48\times20.43^2=208.36$$

$$V_{A\leftarrow B/b}=2p_2\,q_2\,A^2_b=2\times(1-0.35)\times0.35\times14.58^2=96.98$$

Dominant genetic variances at the two QTLs:

$$V_{D\leftarrow A/a}=p^2_1\,q^2_1\,\Delta^2_a=(1-0.48)^2\times0.48^2\times(-49.28)^2=151.30$$

$$V_{D\leftarrow B/b}=p^2_2\,q^2_2\,\Delta^2_b=(1-0.35)^2\times0.35^2\times(-37.72)^2=74.03$$

Epistatic variance of various kinds between the two QTLs:

$$V_{AA\leftarrow(A/a)(B/b)}=4p_1\,q_1\,p_2\,q_2\,[AA_{ab}]^2=4\times(1-0.48)\times0.48$$
$$\times1-0.35)\times0.35\times8.55^2=16.60$$

$$V_{AD\leftarrow(A/a)(B/b)}=2p_1\,q_1\,p^2_2\,q^2_2\,[A\Delta_{ab}]^2=2\times(1-0.48)\times0.48$$
$$\times1-0.35)^2\times0.35^2\times(-22.29)^2=12.92$$

$$V_{DA\leftarrow(A/a)(B/b)}=2p^2_1\,q^2_1\,p_2\,q_2\,[\Delta A_{ab}]^2=2\times(1-0.48)^2\times0.48^2$$
$$\times(1-0.35)\times0.35\times(-20.63)^2=12.08$$

$$V_{DD\leftarrow(A/a)(B/b)}=p^2_1\,q^2_1\,p^2_2\,q^2_2\,[\Delta\Delta_{ab}]^2=(1-0.48)^2\times0.48^2$$
$$\times(1-0.35)^2\times0.35^2\times53.77^2=9.36$$

Table 2 The estimates for the genetic variance components at the two QTLs of the largest effects for second-year volume growth in the intra- and interspecific crosses of *P. tremuloides* and *P. tremula*. The proportions are given in the parentage

Genetic variance components		Intra- and interspecific hybrid population		
		Intraspecific *E*	Intraspecific *E′*	Interspecific *E*×*E′*
Additive	Locus *A*/*A′*/*a*	208.36 (0.36)	292.61 (0.33)	209.67 (0.13)
	Locus *B*/*B′*/*b*	96.98 (0.17)	478.65 (0.54)	138.65 (0.08)
	Total	**305.34** (**0.52**)	**771.26** (**0.87**)	**348.32** (**0.21**)
Dominant	Locus *A*/*A′*/*a*	151.30 (0.26)	64.12 (0.07)	314.49 (0.19)
	Locus *B*/*B′*/*b*	74.03 (0.13)	0.05 (0.00)	269.34 (0.16)
	Total	**225.33** (**0.39**)	**64.17** (**0.07**)	**583.83** (**0.35**)
Epistatic	Add.×Add.	16.67 (0.03)	45.72 (0.05)	240.17 (0.14)
	Add.×Dom.	12.92 (0.02)	0.02 (0.00)	198.20 (0.12)
	Dom.×Add.	12.08 (0.02)	10.02 (0.01)	154.43 (0.09)
	Dom.×Dom.	9.36 (0.02)	0.00 (0.00)	146.71 (0.09)
	Total	**51.03** (**0.09**)	**55.76** (**0.06**)	**739.51** (**0.44**)

The genetic variance components for the intraspecific hybrids of *E′* and mixed intra- and interspecific hybrids were also calculated (Table 2). Epistatic variance components are not prevalent in either intraspecific hybrid population, with the proportions of epistatic components to the total genetic variance being 0.06–0.09. Substantial contributions of epistasis to the genetic variance (0.44) were documented in the mixed hybrid populations of the two species. Although *P. tremuloides* and *P. tremula* naturally have no overlapping distributions as a result of geographic isolation, they are two evolutionarily related species and easily infertile (Mitton and Grant 1996). Strong epistatic components maintained by their hybrid populations may be the genetic cause of growth heterosis and also provide some "fuel" to form new species in nature.

Discussion

The theory that epistasis may play a creative role in evolution and speciation is one of the major contributions to evolutionary biology by Sewall Wright (Wright 1980; Carson and Templeton 1984; Provine 1986; Barton and Turelli 1989; Wade 1992). According to this theory, natural selection acts to retain favorably interacting gene combinations. As a result of the highly integrated nature of the genome, evolution may lead to the production of what Dobzhansky (1970) has termed "coadapted" gene complexes. By contrast, Fisher (1930) argued that natural selection acts primarily on single genes rather than on gene complexes. In this case, natural selection favors alleles that elevate fitness, on average, across all possible genetic backgrounds within a lineage. The test for these two theories has been found to be difficult because no powerful means has been developed to estimate epistasis. Much of the work on epistasis has been only restricted to theoretical inferences with little convincing empirical support. Also, the estimation of epistatic genetic variance components in previous studies may be problematic due to inappropriate design and analysis in experiments on random genetic drift (Lynch 1988). The validation and test of Wright's evolutionary theory critically rely on the development of a robust statistical approach to estimating epistasis.

Both Wright's and Fisher's theories assume that adaptive evolution proceeds independently within distinct evolutionary lineages. However, natural hybridization between divergent lineages may also be an important evolutionary force, a phenomenon that has been well-documented in many wind-pollinated, allogamous species (Stebbins 1959; Whitham 1989; Rieseberg 1985). In many cases, natural hybrids display different morphological, physiological and developmental features from their parents as a result of new combinations of coadapted gene complexes. Studies of hybrid zones can identify genetic factors causing the changes and biodiversity of populations. However, it is impossible to carry out such studies without the estimation of epistatic interactions derived from the new combination of genes. Many researchers have attempted to document the epistatic evidence for hybrid speciation by means of molecular markers in many species, such as *Helianthus anomalus*, *H. deserticola*, and *H. paradoxus* (Rieseberg et al. 1990, 1995, 1996; Rieseberg 1991), *Iris nelsonii*, *I. fulva* and *I. brevicaulis* (Arnold et al. 1990, 1993; Burke et al. 1998) and *Pinus* densata (Wang and Szmidt 1994). However, these studies have not associated molecular markers with phenotypic traits in hybrid zones and have an inability to estimate the contributions of different kinds of epistatic variances to the total genetic variance. As a result, despite the rapid accumulation of molecular data, evidence for the role of epistasis in forming hybrid zones is still not convincing.

Statistical methods for estimating epistasis were based on traditional intraspecific mating designs (Cockerham 1954). Their application to interspecific hybrids is frustrated by the underlying assumptions. By modifying a factorial mating design, Wu and Li (1998) provided a general method for extending estimates of the overall genetic structure of a population to the individual QTL level. Using this method, one can estimate the number of QTLs,

the magnitudes of their effects and allelic frequencies at different QTLs in segregating populations. In this paper, we expand the method to estimate the contribution of various genetic components to quantitative genetic variation in an intraspecific hybrid population and a mixed intra- and interspecific hybrid population. The formulas derived for calculating epistatic variance components in the mixed hybrid population can be directly used to study the genetic architecture of hybrid zones. The procedures described in this paper have been illustrated using an example derived from crosses between *Populus tremuloides* and *P. tremula.* Although these two aspen species are very similar in many morphological characteristics, their interspecific hybrids display pronounced heterosis in stem growth (Heimburger 1936; Einspahr 1984; Li and Wu 1996). Wu and Li's (1999) joint analyses of intra- and interspecific hybrids indicate that although a polygenic model could affect variation in growth traits between these two species, a few key QTLs likely play a more important role in affecting such variation than many others. In this paper, the two QTLs of the largest effects were used to estimate the contribution of epistasis to the total genetic variance in growth traits. It was found that epistasis only explains a small proportion of the genetic variance (<0.10) in the pure intraspecific hybrid progeny of *P. tremuloides* or *P. tremula* but contributes substantially (0.44) to the genetic variance in the mixed progeny population of these two parental species. Thus, strong epistatic components in the hybrid populations may form the genetic basis of heterosis that is commonly observed between these two species. Meanwhile, it is likely that epistasis can provides some fundamental "fuel" to generate new species between these two species. Only when the contribution of epistasis to population genetic variance is specified, like here in aspens, can disputes about the potential role of interlocus genetic interaction in evolution and speciation be resolved [e.g. the Fisher School (Barton and Charlesworth 1984) vs. the Wright school (Carson and Templeton) 1984].

Our method is not dependent on molecular information, which is advantageous on one hand but also disadvantageous on the other hand. One of the advantages is its cheapness and rapidness relative to a molecular experiment in which genotyping a large progeny population required for our method by means of molecular technologies is very costly and time-consuming. More importantly, results obtained from this method can provide insights into the design of a molecular experiment aimed at a more precise estimate of epistasis. A major disadvantage of this method is its dependence on four genetic assumptions, although the assumptions used have been well-justified in current molecular experiments (see Wu and Li 1999). In addition, this method does not enable one to estimate the chromosomal positions of those QTLs that display strong epistatic interactions. The incorporation of molecular markers into this method can overcome these advantages, and their uses to estimate the role of epistasis have been considered by a number of researchers (Shearn 1989; Cheverud and Routman 1995, 1996; Doubley et al. 1995; Lark et al. 1995; de Visser et al. 1997b). The connection between mathematical analyses like this and molecular information is under way in our Program.

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