

Variance Analysis of Immunoglobulin Alleles in Natural Populations of Rabbit (*Oryctolagus cuniculus*): The Extensive Interallelic Divergence at the *b* Locus Could Be the Outcome of Overdominance-Type Selection

Wessel van der Loo

Vrije Universiteit Brussel, St-Genesius-Rode, B-1640 Belgium

Manuscript received September 3, 1991

Accepted for publication April 12, 1993

ABSTRACT

Population genetic data are presented which should contribute to evaluation of the hypothesis that the extraordinary evolutionary patterns observed at the *b* locus of the rabbit immunoglobulin light chain constant region can be the outcome of overdominance-type selection. The analysis of allele correlations in natural populations revealed an excess of heterozygotes of about 10% at the *b* locus while heterozygote excess was not observed at loci determining the immunoglobulin heavy chain. Data from the published literature, where homozygote advantage was suggested, were reevaluated and found in agreement with data here presented. Gene diversity was evenly distributed among populations and showed similarities with patterns reported for histocompatibility loci. Analysis of genotypic disequilibria revealed strong digenic associations between the leading alleles of heavy and light chain constant region loci in conjunction with trigenic disequilibria corresponding to a preferential association of *b* locus heterozygosity with the predominant allele of the heavy chain *e* locus. It is argued that this may indicate compensatory or nonadditive aspects of a putative heterozygosity enhancing mechanism, implying that effects at the light chain might be more pronounced in populations fixed for the heavy chain polymorphism.

THE mechanisms sustaining genetic diversity in populations and their relevance to adaptive evolutionary change have been major issues in population biology and evolutionary theory (LEWONTIN 1974; CLARKE 1979). While the theory of "Neutral Mutation-Random Drift" (KIMURA 1968; KING and JUKES 1969) can explain most observations on genetic variation, there are notable exceptions such as polymorphisms of some genetic loci involved in immune response ("immunity loci"). The diversity patterns at loci of the major histocompatibility complex (MHC) have been studied in detail [reviewed in HUGHES and NEI (1988, 1989)]. They are characterized by (1) high heterozygosity levels (*i.e.*, many alleles); (2) evenly distributed allele frequencies; (3) many alleles in strong linkage disequilibrium; (4) extensive divergence between alleles; (5) high proportions of amino acid altering *vs.* synonymous nucleotide substitutions and (6) persistence (and coalescence) times of alleles that are longer than speciation times (*i.e.*, *trans*-species polymorphisms in KLEIN (1986) and FIGUEROA, GÜNTHER and KLEIN (1988)].

In the European rabbit (*Oryctolagus cuniculus*), alleles of the *b* locus of the constant c_{k1} region of the immunoglobulin (Ig) light (L-) chain (OUDIN 1960) can differ at up to 40% of their amino acid positions (REISFELD, DRAY and NISONOFF 1965) [reviewed in MAGE (1987)]. In spite of this extreme degree of

interallelic divergence, genome analyses have confirmed the rabbit *b* locus allotypes as true alleles of a single-gene locus (EMORINE *et al.* 1984; MATTHYSSENS *et al.* 1985). The analysis of the patterns of nucleotide substitutions at *b* locus genes revealed close analogies to those reported for MHC loci, at both the qualitative and quantitative level (VAN DER LOO and VERDOODT 1992). According to NEI and coworkers, such patterns constitute evidence for overdominant selection (HUGHES and NEI 1988, 1989; TAKAHATA, SATTA and KLEIN 1992).

It should be emphasized that, if overdominance is expected to enhance allelic divergence, this is not because of the relative increase in viability of heterozygote individuals, but because of the way this increase is bound to affect allele distribution in populations (CROW and KIMURA 1970). Any mechanism mimicking the effect of viability selection on allele distribution is liable to influence evolutionary patterns as expected with such selection. As an example, gene divergence at certain mouse MHC loci could be due to MHC allele-specific mating choice which, at least in seminatural populations, causes heterozygous excess (POTTS, MANNING and WAKELAND 1991). The purpose of the present study is not to show that the rabbit *b* locus is exposed to viability selection but to verify whether in natural populations, nonrandom distributions of allele exist that might explain the very unusual

evolutionary patterns as inferred from DNA sequence comparisons.

On the Iberian peninsula—which is probably the aboriginal range of the European rabbit—at least 11 alleles have been distinguished and levels of heterozygosity were similar to those found at murine and human *MHC* loci (VAN DER LOO, FERRAND and SORIGUER 1991). High numbers of alleles, although a predictable outcome of diversity enhancing selection (LI 1978; LEWONTIN, GINZBURG and TULJAPURKAR 1987), make it more difficult to assess selection force from population studies (NADEAU *et al.* 1988). In the recent distribution area of the species (which includes Western Europe, Great Britain, Australia), the wild populations generally show not more than two “effective” *b* locus alleles. In this simplified genetic context, an analysis of gene correlations and gene diversity at loci of the Ig heavy (H-) chain and L-chain was carried out.

MATERIALS AND METHODS

Analysis of genetic variation: The parameters of population structure were estimated from variance components in hierarchically subdivided populations following COCKERHAM (1973) and WEIR and COCKERHAM (1984). As we are interested mainly in allele correlations within individuals, we present results based on weighted mean squares (COCKERHAM 1973). The correlations between genes are (1) within individuals: F ; (2) within individuals within divisions: f ; and (3) of different individuals within the same division: θ . F , f and θ are analogous to WRIGHT's (1951, 1978) F_{IT} , F_{IS} and F_{ST} , respectively.

The relative effect of subdivisions (S) within divisions (D) was estimated by the correlation between a pair of genes between individuals within the same subdivision: θ_1 , and by the correlation between a pair of genes between subdivisions within in the same division: θ_2 . F^{DS} , f^{DS} and θ^{DS} correspond to f_3 , f_1 and f_2 in COCKERHAM (1973) and are estimates of F , f and θ for subdivisions S where effects due to differences between divisions D are ignored. Four levels of subdivision were defined: $A > P > S > W$. They correspond to subdivisions (S) of sample populations or localities (P) from geographically separate areas (A). One sample population was further subdivided into warrens (W). This is indicated by superscripts A , P , S or W such that f^A is the correlation of genes within individuals within areas; θ^{PS} is the correlation between genes of different individuals within subdivisions within populations and so on.

Analysis of genotypic disequilibria at two loci: Complete analysis of two loci disequilibria (WEIR and COCKERHAM 1989) was performed as in WEIR (1990). For two loci b and e , with alleles B, b and E, e , respectively, the total deviation of genotype frequencies from Hardy-Weinberg equilibrium (HWE) was resolved into components which are due to (1) the one locus disequilibria: D_b and D_e , (2) the digenic disequilibrium: Δ_{be} , (3) the trigenic disequilibria: D_{bBE} and D_{BeE} and (4) the quadrigenic disequilibrium: D_{bbe} . The trigenic disequilibria estimate the associations of genotypes at one locus with a particular allele of the other locus, the quadrigenic disequilibrium represents the part of the total disequilibrium not due to previous components. D_b is analogous to COCKERHAM's (1973) variance component due to differences between individuals and an approximation of the correlation F_b can be drawn from $F_b = D_b/p_b p_b$ (for the

locus indicated by the subscript, here the b locus). Note that $D_{bBE} = -D_{bbe} = 2D_{bbe} = -2D_{bbE}$, etc. A computer program was generously made available by BRUCE S. WEIR, North Carolina State University.

Localities: Field collections were made in Southwest Australia (CH, CN, QU), Eastern Australia (BB, BW, MO, UR, GC), Great Britain (GL, Scotland; DO, Humberland; HI, Northumberland; PO, Sussex) and continental Europe (NH, North Holland; WE, Belgian Limbourg; CL, Ile de France). The sample was previously studied for nonrandom associations between the L-chain and H-chain allotypes (VAN DER LOO *et al.* 1987).

Sample collection: Two different methods of collecting individual rabbits were used. (1) *One-off* samples refer to samples collected by shooting rabbits within a time period of a few days. (2) *Samples with known spatial distribution* refer to localities where populations were surveyed for 2–3 years by continuous live-trapping, with individuals marked and released (Grassy Creek (GC) and Urana (UR), South West Australia, and Noord-Holland (NH), Netherlands). All rabbits were weighed. Their age in days can be estimated as 1/10 of their weight in grams (SOUTHERN 1940). Only the data for those weighing more than 600 g were considered here ($N = 1539$). The Australian populations were collected and monitored by B. J. RICHARDSON, Australian National University, Canberra, those from Holland by M. WALLAGE-DREES, Vrije Universiteit Leiden, the Netherlands, during specific research. Blood was collected once, at first capture. Data on warren membership were available for these samples.

Alleles studied: The samples were tested for the following allotypic markers [for review see MAGE (1986)]: *b* locus: allotypes b_4 , b_5 , b_6 , b_9 , which are associated with multiple amino acid differences throughout the constant region of the L-chain of the Kappa-1 (c_{k1}) class. *a* locus: allotypes a_1 , a_2 , a_3 corresponding to multiple amino acid interchanges within the invariable part (framework) of the “variable” region of the Ig H-chain (v_H). *d* locus: allotypes d_{11} and d_{12} , associated with a Met/Thr interchange in the Hinge region of the Ig H-chain of the Gamma isotype (IgG). *e* locus: allotypes e_{14} and e_{15} , associated with a Thr/Ala interchange in the second domain of the same IgG H-chain. The d and e loci are very closely linked and show a very strong linkage disequilibrium: the $d_{11}e_{14}$ combination has never been observed. We can therefore consider the de locus with three alleles $d_{11}e_{15}$, $d_{12}e_{15}$ and $d_{12}e_{14}$.

Analysis of the allotypes: Antisera against a particular allotype were raised in rabbits which were negative for this allele following KELUS and GELL (1967). Phenotypes of the individuals were determined by immunodiffusion, as described previously (VAN DER LOO, FERRAND and SORIGUER 1991).

RESULTS

The semiquantitative properties of the immunodiffusion assay system used permitted classification of the reactions of test sera as O, I, J, K, L, P respectively, from absolutely negative (O), to strong (P). Virtually all I and J reactions—which correspond to immunoglobulin concentrations below 0.1 mg/ml—were found in very young rabbits (weighing less than 500 g) which were not included in the analysis. Sera of rabbits weighing 600 g or more showed the presence of at least one and never of more than two alleles of the loci tested. All precipitation reactions were very clear

TABLE 1
Correlations between *Ig* alleles in subdivided populations

Correlation ^a of genes and Chi-square	Allele							
	<i>b4</i>	<i>b5</i>	<i>b9</i>	<i>a1</i>	<i>a2</i>	<i>a3</i>	<i>d11</i>	<i>e14</i>
Within individuals								
F^P	0.132	0.034	0.225	0.115	0.180	0.141	0.148	0.069
χ^2	16.9	0.6	58.1	16.3	43.9	26.4	29.8	6.3
Within indiv. within divisions								
f^A	0.092	0.008	0.155	0.066	0.156	0.115	0.138	0.065
χ^2	12.7	0.1	19.4	70.2	34.4	19.7	29.3	6.4
f^P	-0.048	-0.053	0.001	0.042	0.112	0.081	0.095	0.040
χ^2	2.5	3.6	0.0	2.3	13.2	8.9	11.8	2.4
f^S	-0.052	-0.056	-0.003	0.040	0.106	0.078	0.084	0.028
χ^2	2.8	3.9	0.0	2.1	11.5	8.1	8.9	1.0
Between indiv. within the same division								
θ^A	0.024	0.020	0.079	0.068	0.025	0.031	0.002	0.000
χ^2 /d.f.	28.3	17.7	192.2	70.2	28.5	33.1	2.5	0.0
θ^P	0.172	0.083	0.224	0.076	0.077	0.065	0.058	0.030
χ^2 /d.f.	43.4	18.5	83.1	15.6	18.2	13.6	12.7	4.0
θ^S	0.154	0.075	0.202	0.068	0.073	0.060	0.062	0.039
χ^2 /d.f.	16.9	7.6	32.4	6.7	8.4	6.4	4.1	1.0

The Chi-square values (χ^2) are COCKERHAM's (1973) χ^2_5 for testing $f = 0$ and $(\chi^2_1 - \chi^2_2)/d.f.$ for $\theta = 0$. For F , χ^2 estimates the probability of Hardy-Weinberg equilibrium within localities.

^a 1539 individuals within either 41 subdivisions (*S*), 16 localities (*P*) or 3 geographic areas (*A*).

TABLE 2
Gene correlations at *Ig* loci in hierarchically subdivided populations

Correlations of genes	Mean over alleles ^a				Mean over loci ^b
	<i>b</i> locus	<i>a</i> locus	<i>d</i> locus	<i>e</i> locus	H-chain
Between individuals within the same subdivision					
θ_1^{AP}	0.1279	0.0785	0.0491	0.0248	0.0637
θ_1^{PS}	0.1421	0.0753	0.0693	0.0423	0.0685
Between individuals of different subdivisions in the same division					
θ_2^{AP}	-0.0412	0.0223	-0.0310	-0.0178	0.0051
θ_2^{PS}	0.1382	0.0713	0.0551	0.0262	0.0605
Within individuals within subdivisions (f_1)					
f^{AP}	-0.0457	0.0708	0.0950	0.0404	0.0703
f^{PS}	-0.0493	0.0677	0.0845	0.0281	0.0642
Between indiv. within subdiv. of the same division (f_2)					
θ^{AP}	0.1624	0.0575	0.0777	0.0418	0.0590
θ^{PS}	0.0045	0.0044	0.0151	0.0165	0.0086
Within indiv. within subdiv. within divisions (f_3)					
F^{AP}	0.1241	0.1242	0.1653	0.0805	0.1252
F^{PS}	-0.0446	0.0718	0.0983	0.0441	0.0723

^a Calculated from sums-over-alleles of weighted variance components (REYNOLDS, WEIR and COCKERHAM 1983).

^b From sums-over-loci of weighted variance components.

and fused perfectly with the positive control, revealing serologic identity with the reference allotypes—apart from a few sera from continental Europe, where a type of cross-reaction with the *a3*-specific antiserum was observed, indicating the presence of an allelic form different from those known in domestic breeds. This variant was pooled with the *a3* allotype. The *b6* allele was observed in only one locality in three individuals (CL): it was pooled with *b5*.

Effect of geographical separation: Sample populations (*P*) were grouped according to geographic

origin (*A*): Australia, Great Britain and Continental Europe. Table 1 presents estimates of correlations between genes of the same allotype for different levels of subdivisions. Mean correlations for alleles per locus or per group of loci are presented in Table 2. Table 3 presents the relative gene frequencies at the different localities and interlocality variances σ^2_h of average within-locality heterozygosity H_P (NEI 1973).

Genetic differences were clearly larger between populations within areas than between areas ($\theta^A \ll \theta^P$ in Table 1 and $\theta_2^{AP} < 0$ in Table 2). Only for the *a1*

TABLE 3
Allele frequencies and interlocality variances of expected heterozygosity levels

Area locality	Allele frequencies								
	2N	b4	b5	b9	a1	a2	a3	d11	e14
Australia									
BB	46	0.848	0.152	0.000	0.370	0.130	0.500	0.520	0.152
BW	78	0.615	0.385	0.000	0.260	0.290	0.450	0.330	0.218
CH	166	0.614	0.367	0.018	0.494	0.012	0.494	0.114	0.235
CN	142	0.739	0.253	0.007	0.460	0.180	0.360	0.090	0.246
MO	30	0.567	0.333	0.101	0.820	0.110	0.070	0.040	0.267
QU	14	0.714	0.286	0.000	0.500	0.000	0.500	0.280	0.143
UN	42	0.619	0.381	0.000	0.440	0.160	0.400	0.120	0.238
UR	154	0.753	0.209	0.038	0.540	0.310	0.150	0.310	0.114
GC	1150	0.727	0.252	0.022	0.582	0.089	0.329	0.298	0.194
Subtotal ^a		0.712	0.267	0.020	0.541	0.119	0.341	0.262	0.200
Great Britain									
DO	178	0.893	0.107	0.000	0.430	0.028	0.539	0.490	0.079
GL	44	0.636	0.364	0.000	0.610	0.050	0.340	0.250	0.204
HI	150	1.000	0.000	0.000	0.450	0.000	0.550	0.310	0.113
PO	234	0.172	0.453	0.375	0.423	0.231	0.346	0.130	0.358
Subtotal ^a		0.625	0.231	0.144	0.449	0.101	0.450	0.279	0.206
Continental Europe									
NH	250	0.646	0.354	0.000	0.160	0.188	0.650	0.270	0.134
WE	106	0.340	0.660	0.000	0.293	0.302	0.387	0.290	0.302
CL	294	0.609	0.289	0.102	0.394	0.211	0.395	0.173	0.259
Subtotal ^a		0.580	0.374	0.046	0.288	0.217	0.492	0.226	0.218
Total ^a	3078	0.667	0.283	0.050	0.469	0.136	0.394	0.255	0.204
Mean and variance of heterozygosities									
		b locus		a locus		de locus			
H_T^b		0.477		0.611		0.598			
H_P		0.410		0.559		0.566			
σ_h^2		0.022		0.008		0.003			

^a Weighted by sample size.

^b H_T is $1 - \sum_i (p_i)^2$, in which p_i is the unweighted mean of allele frequencies p_{il} ; H_P is the unweighted mean over localities l of the expected heterozygosity levels $H_l = 1 - \sum_i (p_{il})^2$, with p_{il} the frequency of allele i at locality l ; σ_h^2 is the variance of H_l .

allele of the a locus was a significant fraction of the interlocality variance due to differences between areas (continents). The genetic homogeneity over areas is particularly striking for the e locus and extends down to the population level (both θ^A and θ^P are very small). Between alleles and loci, differences in θ can be large (see chi-square values in Table 1).

Effect of subdivision of populations: The correlations in Table 1 were calculated as if subdivisions were made for all sample populations although 44% of the total sample consisted of undivided *one-off* populations. Subpopulations (S) relate to geographic divisions within the collection sites, except for one locality (PO in Table 3), where three annual *one-off* collections were made. Between subdivisions of the same populations, genetic differences were very small. Values of parameters θ_2^{PS} , which estimate the effect of subdivision, could, for some alleles, be explained sampling error alone. The comparisons between θ^{AP} and θ^{PS} (Table 2) are relevant here.

Heterozygote excess or homozygote deficiency:

Analyses at the population level indicate a consistent excess of heterozygotes at the b locus, as implied by the negative correlations f . For $b5$, f was positive for only 3 of the 16 localities. Although the values of f in Table 1 are not significantly negative ($f^P_{b5} = -0.053$, $\chi^2 = 3.6$), they clearly differ from the positive values at the other loci. Of 41 subdivisions, 10 showed an excess of $b4$ heterozygotes with $\chi^2 > 1$, while only 4 showed an excess of $b4$ homozygotes with $\chi^2 > 1$. If we write this distribution as $b4$: 10-/4+, then we obtain for the other alleles: $b5$, 13-/6+; $b9$, 0-/1+; $a1$, 4-/9+; $a2$, 1-/12+; $a3$, 4-/10+; $d11$, 2-/13+; $e14$, 7-/6+. The evaluation of correlations between genes of the same locus within individuals (*i.e.*, of homozygote or heterozygote excess) depends greatly upon our knowledge of genetic differences between subareas within localities as this permits to account for Wahlund effect. We have therefore analyzed separately the fraction of the sample for which data on the spatial distribution of the individuals within localities were available.

TABLE 4
Correlations of *Ig* alleles in three populations subdivided in neighborhoods

Subdivided population correlations ^a	Allele							
	<i>b4</i>	<i>b5</i>	<i>b9</i>	<i>a1</i>	<i>a2</i>	<i>a3</i>	<i>d11</i>	<i>e14</i>
θ_2	0.008	0.016	0.006	0.197	0.102	0.172	-0.005	0.006
θ_1	0.014	0.025	0.037	0.202	0.113	0.181	0.017	0.039
θ^{PS}	0.007	0.009	0.031	0.007	0.012	0.011	0.021	0.034
F^{PS}	-0.088	-0.108	-0.019	-0.007	0.102	0.046	0.122	0.047
f^{PS}	-0.096	-0.117	-0.052	-0.013	0.091	0.036	0.103	0.014
χ^2b	6.7	9.9	0.0	0.1	4.4	0.8	7.6	1.7

^a 1554 genes within: 777 individuals, 25 subdivisions (genetic neighborhoods), 3 populations.

^b χ^2 values were calculated for f^S , which is identical to f^{PS} .

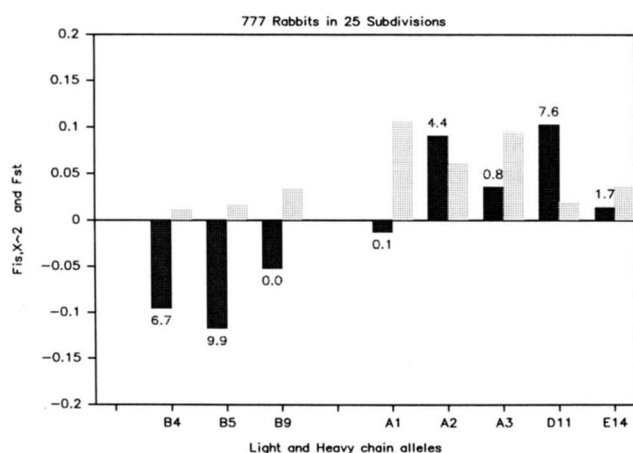


FIGURE 1.—*Ig* allele correlations F_{IS} (f) and F_{ST} (θ) in 25 neighborhoods. Values of F_{IS} (dark) and F_{ST} (light) are presented for the three localities where the Wahlund effect could be accounted for. Chi-square values are indicated for F_{IS} . There is a significant excess of heterozygotes at the *b* locus whereas the distribution of the heavy chain indicates Hardy Weinberg equilibrium or homozygote excess.

Analysis of samples with known spatial distribution: At GC (Grassy Creek, 37% of the total sample), about 80% of the population was sampled and associations with warrens recorded. The total area was subdivided into 11 subareas. Such information was also available for population UR (Urana) and to a lesser extent for population NH (North Holland) where much smaller fractions of the populations were sampled. We do not have information on the spatial distribution of the individuals in the other samples. In Table 4 correlations of genes are estimated for these three populations. In this sample, the heterozygote excess at the *b* locus was significant ($F_{b5}^P = -0.09$; $f_{b5}^P = -0.11$, $P < 0.005$). (Note: If chi-square values were calculated according to BARKER, EAST and WEIR (1986), significance levels for $f < 0$ were slightly higher.) By contrast, H-chain alleles often show positive f values (Figure 1). Subdivision had little if any effect (θ^{PS} and θ_1^{PS} - θ_2^{PS} close to zero), indicating genetic homogeneity within localities. Therefore, f^S hardly differs from f^P ($f_{b5}^S = -0.12$; $P < 0.002$).

In Table 5 the data on population GC were treated

separately. The total study area covered about 1 km² and the widest gap without active burrows measured less than 250 m, the largest distance between two burrows was 1600 m. For the present analysis the original number of warrens was reduced to 55 by grouping proximate burrows (within 15 m) in order to have at least 5 samples per warren. Apart from *e* locus alleles ($\theta^S = 0.04$, $P < 0.03$, not shown), genetic differentiation between subareas and between warrens was not significant and sometimes slightly lower than expected from sample variance alone ($\theta^{SW} < 0$). In the undivided GC sample, F was significantly negative for the *b* locus ($F = -0.10$, $P = 0.01$).

Analysis of the one-off samples and of published data: The *one-off* samples—the set complementary to the three samples analyzed above—consists of 762 animals in the remaining 13 population samples. Here, the estimates of f for the *a* locus revealed important homozygote excess: $f_{a \text{ locus}} = 0.11$ (not shown). The f values for the *b* locus alleles were in perfect agreement with HWE: $f_{b \text{ locus}} = 0.00$ (not shown). HERD and EDMONDS (1977) published the *a* and *b* locus genotype frequencies for 25 districts of South-Eastern Australia, grouped into 6 areas. We have estimated for 5384 rabbits from 25 districts the population parameters according to COCKERHAM (1973). The difference between the correlations at the *b* locus versus those at the *a* locus was similar to what was observed in *one-off* populations (Table 6).

Association of heterozygote excess at the L-chain *b* locus with H-chain genotypes: When COCKERHAM's variance analysis was applied to samples grouped according to their H-chain genotypes, the strongest variation in *b* locus correlations was found between classes of *e* locus genotypes. For simplicity, the *b* locus is presented as a two-allele locus with alleles *b* and *B* (*b5* and *non-b5*, respectively). Alleles *e* and *E* designate the *e14* and *e15* alleles, respectively (Tables 7 and 8). In Table 7, the heterozygote excess at the *b* locus appears to be associated in essence with *E* homozygosity ($f_{b(EE)} = -0.09$, $\chi^2 = 6.3$). Such negative correlation is not observed amongst heterozygote *eE* rabbits ($f_{b(eE)} = -0.01$, $\chi^2 = 0.1$).

TABLE 5
Correlations of *Ig* alleles at Grassy Creek (GC)

Subdivisions divided into Warren correlation of genes	Allele							
	<i>b4</i>	<i>b5</i>	<i>b9</i>	<i>a1</i>	<i>a2</i>	<i>a3</i>	<i>d11</i>	<i>e14</i>
θ_2	-0.009	-0.008	0.032	-0.014	-0.007	-0.017	-0.012	0.019
θ_1	0.002	0.003	0.024	0.054	0.028	0.044	0.049	0.086
θ^{SW}	0.011	0.010	-0.008	0.067	0.035	0.059	0.060	0.068
F^{SW}	-0.069	-0.100	-0.048	-0.010	0.080	0.056	0.110	0.044
f^{SW}	-0.080	-0.111	-0.040	-0.082	0.047	-0.003	0.053	-0.026
χ^2	3.3	6.5	0.5	3.1	0.9	0.0	1.3	0.3

1150 genes within: 575 individuals, 55 warrens, 11 subdivisions.

TABLE 6
COCKERHAM'S correlations of *Ig* alleles in Victoria (Australia)

Correlations	Allele							
	<i>b4</i>	<i>b5</i>	<i>b9</i>	<i>a1</i>	<i>a2</i>	<i>a3</i>	<i>b</i> locus	<i>a</i> locus
θ_2	0.0154	0.0126	0.0124	-0.0007	0.0076	0.0023		
θ_1	0.0414	0.0389	0.0345	0.0595	0.0198	0.0532	0.04	0.04
θ^{DP}	0.0264	0.0266	0.0225	0.0602	0.0123	0.0510		
F^{DP}	0.0711	0.0860	0.0591	0.1954	0.1456	0.1813		
f^{DP}	0.0459	0.0611	0.0375	0.1438	0.1350	0.1373	0.06	0.14

5384 rabbits in 25 localities in 6 divisions [genotype data from HERD and EDMONDS (1977, Table 1-2)].

TABLE 7
Correlations of *b5* alleles in rabbits with different *e* locus genotypes

<i>b</i> Locus correlations within <i>e</i> locus classes	<i>e</i> Locus genotype classes and no. of individuals		
	<i>e15/e15</i> 991	<i>e14/e15</i> 468	<i>e14/e14</i> 80
F^s	0.017	0.042	0.121
χ^2	0.4	0.7	1.0
f^s	-0.090	-0.011	0.029
χ^2	6.3	0.1	0.3
θ^s	0.067	0.053	0.095
χ^2 /d.f.	4.6	2.6	2.5

1539 rabbits were grouped according to their *e* locus genotype. In each genotype class, correlations *F*, *f* and θ of *b5* genes were estimated for the sample subdivided into 41 geographical subdivisions.

This genotypic disequilibrium was investigated following WEIR (1990). Table 8 displays the numbers of the nine phenotypes observed, compared to the expectations in case monogenic and digenic interactions (*i.e.*, inbreeding and linkage disequilibrium) were the only sources of disequilibrium. It shows that under tri- and quadrigenic equilibrium, homozygote—rather than heterozygote—excess at one locus is expected to be largest within the group of individuals homozygous for the leading (*i.e.*, more common) allele of the other locus. This effect of the digenic associations is countered by trigenic associations which correspond to an increased level of heterozygote *bB* genotypes among

rabbits expressing the *E* allotype and of *eE* genotypes among rabbits expressing the *B* allotype. The quadrigenic disequilibrium does not contribute to this increase and is not significant. The observed incidence of *BbEE* genotypes is 5% higher than expected under trigenic equilibrium. The overall trigenic effect may be significant ($\chi^2 > 5$; $P < 0.05$) and is due for 90% to *D_{BbE}*. It can be shown that this nonrandom association is the accumulation of convergent effects within localities (Table 7) and between localities (Figure 2).

DISCUSSION

Distribution of gene diversity among localities and areas: For the four *Ig* loci and the ten alleles studied, the mean θ across geographic areas was estimated as $\theta^A = 0.023$, which implies a degree of genetic differentiation similar to what was observed for enzyme loci between populations of house mice (*Mus musculus*) in barns within Texas farms, or between populations of the land snail *Helix aspersa* within small city blocks (SELANDER and KAUFMAN 1975; AVISE, SMITH and SELANDER 1979). It is also close to the values reported here for parameters estimating the degree of differentiation between subdivisions of the same population (θ^{PS} in Table 4) or between warrens of a locality, where the genetic homogeneity across subareas was confirmed (θ^{SW} in Table 5). More genetic differentiation was found between localities of the same continent than between continents. However, the interlocality variation in allele frequency was much

TABLE 8
Analysis of HWE with two-locus two-allele interactions

	EE	Ee	ee	Total	Coefficients of disequilibrium	
Observed genotype frequencies						
BB	554	216	28	798	p_b	0.283
Bb	379	198	35	612	p_e	0.204
bb	58	54	17	129		
Sum	991	468	80	1539	D_b	0.0039 $\chi^2 = 0.6$
					D_e	0.0104 $\chi^2 = 6.6$
					Δ_{eb}	0.0289 $\chi^2 = 36.6$
Per <i>e</i> locus genotype class						
p_b	0.250	0.327	0.437	0.283	D_{BbE}	0.0066 $\chi^2 = 4.3$
D_b	-0.0038	0.0085	0.0265	0.0039	D_{BeE}	0.0036 $\chi^2 = 1.4$
F_b	-0.020	0.039	0.108	0.019	D_{bbe}	0.0006 $\chi^2 = 0.3$
Expectations at tri- and quadrigenic equilibrium						
BB	565.31	203.59	29.11	798	p_b	0.283
Bb	362.00	211.58	38.42	612	p_e	0.204
bb	63.69	52.83	12.48	129		
Sum	991.00	468.00	80.00	1539	D_b	0.0039 $\chi^2 = 0.6$
					D_e	0.0104 $\chi^2 = 6.6$
					Δ_{eb}	0.0289 $\chi^2 = 36.6$
Per <i>e</i> -locus genotype class						
p_b	0.247	0.339	0.396	0.283	D_{BbE}	0.00
D_b	0.0033	-0.0020	-0.0038	0.0039	D_{BeE}	0.00
F_b	+0.018	-0.009	-0.007	0.019	D_{bbe}	0.00

The coefficients of disequilibrium were determined following WEIR (1990). *b* and *B* designate the *b5* and *nonb5* alleles of the *b* locus, *e* and *E* the *e14* and the *e15* alleles of the *e* locus. Expected frequencies are shown for a hypothetical sample with allele frequencies, mono- and digenic disequilibria as in the sample under study. It shows that the digenic disequilibrium should decrease the correlations of *b* locus alleles among rabbits expressing the *e14* allele and can clearly not explain the observation that in the sample studied, heterozygous excess tend to be strongest among homozygous *e15* rabbits (Table 7). This is entirely due to trigenic associations which correspond to a preferential association of heterozygosity at one locus with the leading allele of the interacting locus.

lower for H-chain loci than for the L-chain locus ($\theta^{AP} = 0.06$ vs. 0.16; Table 2).

In the area covered in this study, rabbits were introduced as semidomesticated breeds during historical times (*i.e.*, after the Roman Conquest). Ancestors of the British rabbits reached England about 1000 years ago (ZEUNER 1963). Australian rabbits are believed to be the descendants of 24 wild rabbits from England which were released in 1895 close to Geelong, Victoria (HOLLAND 1923; KIDDLE 1961; FENNER and RATCLIFFE 1965). In all areas studied here, rabbits have been very abundant before the introduction, 40 years ago, of Myxoma virus, which at the earlier stage of the epizootic, killed some 90–99% of individuals in exposed populations (FENNER 1953; LLOYD 1981). Myxomatosis is still regarded as a major mortality factor in the areas studied here and has been used as a pest control agent (MYERS, MARSHALL and FENNER 1954; ROSS and TITENSOR 1981 1986).

The lack of genetic differentiation between the three major areas, which is particularly striking for the *e* locus alleles, is therefore surprising as strong founder effects are to be expected and the effective population sizes of rabbits are also under normal conditions rather small [90–250 according to DALY (1979) and to RICHARDSON (1981)].

NADEAU *et al.* (1988) reported that the *H-2* polymorphisms were more uniformly distributed amongst

wild mice populations than are enzyme polymorphisms and this was presented as an indication of the adaptive value of the *H-2* variability. They observed that, while similarity networks based upon allozymes gave a reliable presentation of the geographic relationship between populations sampled, networks based upon *H-2* polymorphisms bore little correspondence to known relationships.

RICHARDSON, ROGERS and HEWITT (1980) estimated genetic distances between Australian, British and French rabbits based upon polymorphic loci of the red cell isozymes and found larger genetic distances between geographic areas than between populations within areas (*i.e.*, the values of θ_2^{AP} should be positive for the enzyme polymorphisms). In contrast, the negative θ_2^{AP} values reported here for the *Ig* loci, would mean that on the average, a population is rather more similar to a population of another area than to one of the same area. The comparison in Table 9 between the *Ig* and *MHC* data confirms that the "surprisingly uniform" distribution of alleles of the *H-2* polymorphism (*i.e.*, the relatively low contribution of differences between localities D_{PA} and between areas D_{AT} to the total gene diversity H_T), clearly also holds for the rabbit *Ig* allotypes.

Such "even distribution" of gene frequencies over geographic areas constitute an interesting characteristic of population diversity which is best quantified

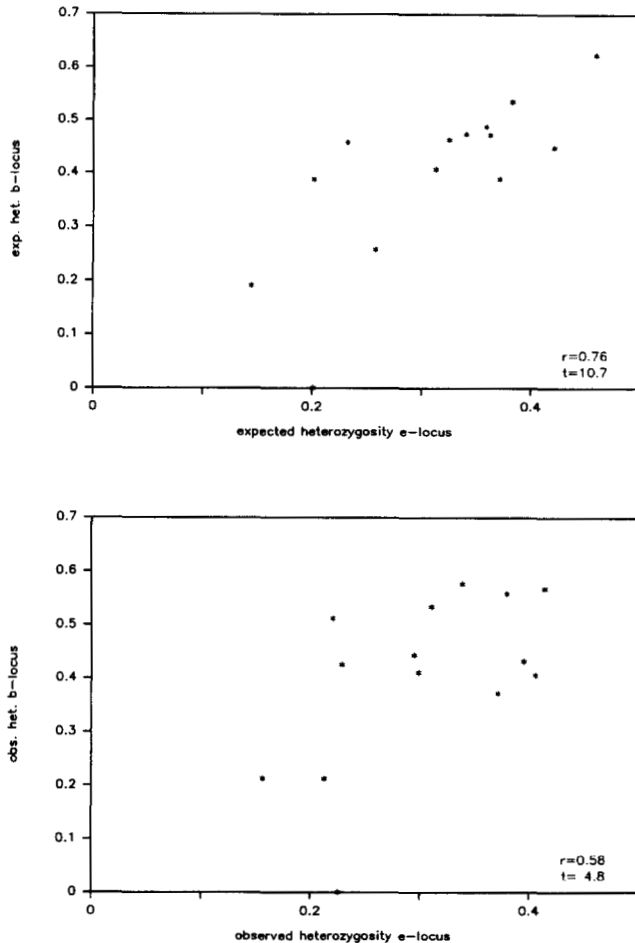


FIGURE 2.—Interlocality correlation between *b* locus and *e* locus heterozygosity. The correlations are visualized between the expected (*top*) and the observed (*bottom*) heterozygote frequencies for samples with more than 20 individuals (unweighted by sample size; d.f. = 12). The significance of the correlation coefficient r is estimated by a t -test as suggested in CROW and KIMURA (1970). The strong correlation in expected heterozygosities reflects the interlocality component of the digenic or "linkage" disequilibrium between these loci. As a consequence of monogenic and trigenic disequilibria, the correlation is less pronounced between the observed heterozygosity levels.

by the parameter θ_2 . Indeed, negative correlations between localities of the same geographical area (relative to the total) are not unexpected if gene frequencies are maintained by some mechanism around a value which is similar for the different areas while at the same time, within smaller breeding units, significant fluctuations around these frequencies occur due to genetic drift or variations in selection pressure. It should be mentioned here that the hierarchy in gene frequencies of *a* and *b* locus alleles [Table 3; cf. Table 6 in VAN DER LOO FERRAND and SORIGUER (1991)] are matched by the systematic hierarchy in the relative expression levels of these alleles in heterozygote individuals. Indeed, each lymphocyte expresses only a single Ig allele [*allelic exclusion*, RITCHIE, BRINSTER and STORB (1984)] and in heterozygote rabbits, there is a systematic imbalance in the relative number of

mature lymphocytes expressing one or the other of the *a* locus and *b* locus alleles (LUMMUS, CEBRA and MAGE 1967). This allelic imbalance can be summarized as: $b_4 > b_5 \gg b_9$, $a_1 > a_2$ and $a_3 > a_2$ and was also observed in wild rabbits studied here, including rabbits from localities PO and WE, where the frequency hierarchy was not respected [KERREMANS (1982); discussed in VAN DER LOO (1987)]. As far as the mechanism underlying the allelic imbalance in gene expression could have an impact on the gene frequencies, we would expect it to favor fixation of those alleles that are preferentially expressed, rather than to impose a hierarchy in gene frequencies as it was proposed by HERD and EDMONDS (1977, 1981). Also, the (digenic) linkage disequilibria which favor the combination of the leading alleles of the *b* and *e* locus is, if anything, expected to cause their fixation if not countered by balancing selection (see below). Therefore the mere fact that the less expressed allelic genes are not lost from the populations constitutes on its own an indication for balancing selection.

Heterozygote excess at the *b* locus: The correlations f and F were consistently lower for *b* locus alleles than for alleles of the H-chain (Table 2). In populations where animals were sampled within what could be called "genetic neighborhoods," the correlation f between *b*₅ genes within individuals was significantly negative ($P < 0.002$; Table 4) whereas no deviation from HWE was apparent for the H-chain alleles. In population GC, treated as one sample, the coefficient of inbreeding F was significantly negative for the *b* locus ($F = -0.09$, Table 5). In 9 of the 11 subdivisions of GC, f and F were smaller than -0.05 both for *b*₅ and *b*₄ (not shown).

Mechanisms such as migration and mating systems could explain heterozygote excess without implying selection (WRIGHT 1978), but in general would affect all loci equally. Yet the f values were often positive for the H-chain loci and always larger than f values for the *b* locus (Tables 1 and 2, 4-6; Figure 1). Although there is no good test of statistic significance (cf. ROBERTSON 1975; EWENS and FELDMAN 1976), this difference in f between loci provides a parameter which is less affected by genetic structuring within sample populations (cf. CAVALLI SFORZA 1966; LEWONTIN and KRAKAUER 1973). For these reasons, the data of HERD and EDMONDS (1977) on Australian rabbits were reexamined.

As shown in Table 6, also in HERD and EDMONDS (1977) samples, the correlations between *b* locus genes were considerably lower than between *a* locus genes. However, f values derived from their data were positive for both loci, which led HERD and EDMONDS to suggest homozygous advantage as the mechanism maintaining allelic diversity of the Ig loci. This discrepancy with our observations could relate to differ-

TABLE 9

Hierarchical analysis of gene diversity at *Ig* loci in rabbit compared to that at *H-2* and allozyme loci in mouse

Locus	Total gene diversity		Distribution of diversity			
	H_T^a $\Sigma v_i^a b$	H_P/H_T $1 - \theta_1^{AP}$	D_{PA}/H_T $\theta_1^{AP} - \theta^A$	D_{AT}/H_T θ^A	$\theta_1^{AP} - \theta_2^{AP}$	θ_2^{AP}
Mouse						
Allozyme ^a	0.23	0.64	0.12	0.24		
<i>H-2</i> ^a	0.59	0.83	0.06	0.10		
Rabbit immunoglobulin allotypes (this study)						
<i>a1</i> ^a	0.50	0.93	0.03	0.04		
<i>a1</i> ^b	0.51	0.91	0.02	0.07	0.04	0.05
<i>a2</i> ^a	0.24	0.93	0.05	0.01		
<i>a2</i> ^b	0.24	0.93	0.05	0.03	0.08	-0.01
<i>a3</i> ^a	0.48	0.94	0.04	0.02		
<i>a3</i> ^b	0.48	0.93	0.04	0.03	0.06	0.01
<i>b4</i> ^a	0.44	0.85	0.14	0.01		
<i>b4</i> ^b	0.45	0.85	0.13	0.02	0.22	-0.07
<i>b5</i> ^a	0.41	0.93	0.06	0.01		
<i>b5</i> ^b	0.41	0.92	0.06	0.02	0.10	-0.02
<i>b9</i> ^a	0.10	0.81	0.14	0.05		
<i>b9</i> ^b	0.10	0.78	0.14	0.08	0.24	-0.02
<i>a</i> locus ^b	0.62	0.92	0.03	0.05	0.06	0.02
<i>b</i> locus ^b	0.48	0.87	0.10	0.03	0.17	-0.04
<i>d</i> locus ^b	0.38	0.95	0.05	0.00	0.08	-0.03
<i>e</i> locus ^b	0.32	0.98	0.03	0.00	0.04	-0.02

The distribution of gene diversity among mouse populations was adapted from data in NADEAU *et al.* (1988), leaving out the differences between subspecies. H_T is total gene diversity or heterozygosity and is analogous to Σv_i^a , the total variance; H_P : diversity within localities; D_{PA} : diversity between localities within geographical regions; D_{AT} : diversity between regions.

^a Following CHAKRABORTY (1974), weighted by sample size.

^b Following COCKERHAM (1973), weighted by sample size.

ences in sampling method: HERD and EDMONDS explicitly avoided the effect of local variation within their samples: "In each district studied, rabbits were collected over long transects to reduce possible sampling errors due to spot collection" (HERD and EDMONDS 1977, p. 316). Therefore, Wahlund effect might be important in their samples. Over 50% of their total sample was collected in just 2 out of the 25 districts. It is likely that larger areas were covered during collection in these two districts as also suggested by their higher values of F (not shown). By limiting the analysis to the 23 smaller sample districts, f was 0.00 at the *b* locus and 0.11 at the *a* locus (not shown). This is identical to what was found in the present study for the *one-off* samples. The variance analysis of HERD and EDMONDS' data therefore corroborates the systematic difference in f between the *b* locus and the H-chain loci: it cannot possibly be due to chance. The data obtained here from extensively sampled populations where Wahlund effect could be accounted for, strongly indicates that this difference is at least in part due to a mechanism leading to heterozygote excess of *b* locus alleles.

Because of the close linkage between genes determining respectively the variable and constant regions of Ig chains, heterozygous excess detected at a constant region locus could, in principle, be a corollary

of heterotic selection at the level of the variable region. Apart from the fact that there seem to be no reports on heterosis of Ig variable region genes (in any species), this is unlikely because alleles of the *a* locus (which controls the variable region of the Ig H-chain) displayed homozygous excess or HWE (Table 2; Figure 1). The *a* locus correlations confirm furthermore that the *b* locus heterozygote excess cannot be due to transplacentally transferred antibodies, as allotypic determinants of maternal Ig of both *a* and *b* locus alleles are known to disappear *simultaneously* from the serum (DRAY 1962). This clearance of maternal Ig is achieved before the age of 8 weeks (DRAY 1972) and from 15 years of experience with the breeding of laboratory rabbits expressly for the *Ig* allotypes, we know for certain that in the age group considered here, our test system reveals the individual *Ig* genotype without any ambiguity. (Note: in the present study the clearance of maternal Ig was also inferred from the serological data on rabbits weighing less than 600 g; not shown.)

Mean heterozygosity and its variance: The mean heterozygosities within-subdivisions H_S (for consistency noted H_P in Table 3) for the *Ig* loci studied here were 0.41, 0.56 and 0.57 for, respectively, the *b*, *a* and *de* loci. This is higher than what is currently observed for protein loci in a wide variety of popula-

tions studies (GOJOBORI 1982) where values of H_S cluster around 0.20. High values of gene diversity H_S are diagnostic for loci where frequencies of segregating alleles are distributed more evenly than expected from random drift (WATTERSON 1978) and may indicate balancing selection (HENDRICK and THOMPSON 1983). Theoretical studies of MARUYAMA and NEI (1981) predict that overdominance would tend to favor high heterozygosity levels, reducing in the process its variance. The interlocality variances in heterozygosity σ^2_h observed here for the *Ig* alleles (0.022, 0.008 and 0.003, Table 3) met MARUYAMA and NEI's (1981) predictions for loci under strong overdominant selection (0.027, 0.008 and 0.008) and were clearly smaller than the values observed for allozyme loci in natural populations by GOJOBORI (1982) which did satisfy predictions for neutral alleles (*i.e.*, 0.050, 0.035 and 0.035 for heterozygosity levels 0.41, 0.56 and 0.57).

For *b* and the *a* locus alleles, the weighted θ^p values range from 0.065 to 0.224 (Table 1) and F_{ST} from 0.085 to 0.21 (easily obtained as $1-H_P/H_T$ from Table 3). This "moderate" to "great" genetic differentiation (HARTL 1981) indicates that the low σ^2_h are not the necessary consequences of low variances in gene frequencies. In a comprehensive study on the distribution patterns of gene diversity, SINGH and RHOMBERG (1987) correlate different modes of variation of the statistics H_T , H_S and F_{ST} to different types of selection regimes. The mode displayed here by the *Ig* alleles is that of loci under putative balancing selection (high H_T). While the relatively high values of θ and F_{ST} for *b9* and *b4* could, according to SINGH and RHOMBERG (1987), suggest diversifying selection, the frequency distributions of *b5* and of the *e* locus alleles are closest to the mode predicted under heterotic selection (low to moderate F_{ST} , low variance in H_S). Our data provide clear indications for heterozygous excess for the *b* locus, but not for the other loci studied.

Genotypic disequilibria between the *b* locus of the L-chain and the *e* locus of the H-chain: Analyses of gene correlations at different loci become notably complex in case of interaction between loci (*cf.* WEIR 1990). As strong "linkage" disequilibria exist between alleles of the *b* and *e* locus (despite the fact that they are located on different chromosomes), putative heterotic effects at the *b* locus could be influenced by the H-chain genotype.

Table 7 reveals that pairs of *b* locus alleles within individuals were negatively correlated within the fraction composed by homozygous *e15* rabbits, while this was not the case in population fractions representing rabbits expressing the *e14* allele. This means that partitioning by *e* locus genotypes was not random with respect to the *b* locus genotype. Considering the strong digenic association between these two loci

within subdivisions (VAN DER LOO *et al.* 1987), this is not surprising: digenic disequilibria can affect the relative distribution of frequencies as well as the (monogenic) correlations at one locus among classes of genotypes of the interacting locus. It was therefore an obvious question to ask whether the apparent association of heterozygous excess with the *e15* homozygosity observed in Table 7 can be explained by the digenic disequilibrium.

The analysis of genotypic disequilibria allows the dissociation of the different levels of gene interaction. Table 8 makes it clear that the digenic disequilibrium can not explain the increase of *b* locus heterozygosity among homozygous *e15* animals. On the contrary, the expected gene correlations at one locus are shown to be *largest* in those animals that are homozygous for the leading allele of the other locus, as a consequence of the positive digenic associations between the leading alleles *B* (*nonb5*) and *E* (*e15*). It appears that this effect of the digenic disequilibrium is opposed by trigenic disequilibria which, in essence, correspond to a preferential association of *b* locus heterozygosity with the leading allele of the interacting *e* locus. As more than 80% of the *e15* genes are at the homozygous state, *b* locus heterozygosity is seemingly correlated with *e* locus homozygosity. Under the assumption that the excess of heterozygotes observed is due to some deterministic process, the positive values of the trigenic associations D_{bBE} and D_{BeE} (Table 8) could indicate that this process is less effective in rabbits that are "already" heterozygous at one locus. This is biologically meaningful: mechanisms favoring diversity at one part of the *Ig* molecule might indeed be attenuated by diversity existing at another part of the molecule.

Compensatory aspects of H- and L-chain diversity: Within this context it is noteworthy that the rabbit *IgG* H-chain constant region is outstanding among mammals by its *lack* of diversity (HAMERS 1987). While in other species there are a number of different c_γ genes (*IgG* subclasses) with complex polymorphisms [*cf.* the H-chain allotypes of human and mouse *Ig*, reviewed in GRUBB (1970) and in HERZENBERG and HERZENBERG (1978)], rabbit has only a single gene coding for c_γ . Only three serological alleles can be distinguished at this locus (*de* locus), which differ by less than four amino acid residues. In most other studied species, such limited allelic variation is a characteristic of the *Ig* L-chain constant region genes. It is possible that the extensive polymorphisms at the rabbit *b* locus and *a* locus compensate for the lack of allelic diversity at the H-chain constant region. The hypothesis that benefits due to allelic diversity at loci composing the *Ig* constant region might be compensatory (nonadditive) merits further research as this should contribute to our understanding of the nature

TABLE 10

Homozygote deficiency d and heterozygote deficiency F at a mouse *MHC* locus and at the rabbit *b* locus

	Number of		H_T	F	d
	Genes	Subdivisions			
Mouse <i>MHC</i> ^a	2278	9	0.79	-0.07	0.27
Rabbit <i>Ig b</i> locus					
Total	3078	16	0.41	-0.05	0.03
Neighborhoods	1554	3	0.42	-0.10	0.08
Grassy Creek	1150	1	0.41	-0.09	0.06
Portugal ^b	374	16	0.73	-0.10	0.27
Iberia + Azores ^b	828	21	0.71	-0.07	0.17

F is $(H_T - H_{ob})/H_T$, in which H_{ob} is the proportion of heterozygotes observed and H_T that expected under Hardy-Weinberg equilibrium. The homozygote deficiency d is $(J_T - J_{ob})/J_T$, in which $J_i = 1 - H_i$ (HEDRICK 1990, 1992). $d = -F H_T/J_T$. For subdivided populations F was estimated by F_{IS} , Wright's coefficient of local inbreeding, and H_T by H_s the mean heterozygosity within subdivisions such that $d = -F_{IS} H_s/J_s$. Note that for the samples from Iberia and Azores, H_T were minimal estimates and F_{IS} determined by an approximation method.

^a Data from POTTS, MANNING and WAKELAND (1991).

^b From Table 2 in VAN DER LOO, SORIGUER and FERRAND (1991).

of the underlying mechanisms. Contrary to what might be expected with mechanisms depending upon external factors, where quantitative and qualitative aspects of allelic diversity might be important, it indicates that the diversity enhancing process may rather rely upon intrinsic factors such as the absence *vs.* presence of antigenic differences (*i.e.*, differences detectable by the immune system).

Such nonadditive or "compensatory" overdominance-type selection should, in the absence of other deterministic effects, favor a negative correlation of heterozygosity levels among the different loci. Within the range of gene frequencies in the studied sample, this implies a negative component of covariance between *b5* and *e14* frequencies. This component should restrain the effect of the positive interlocality correlation between *b* and *e* locus heterozygosity levels which is inherent to the digenic association (Figure 2) and which, otherwise, should favor the fixation of the leading alleles. Thus, while the positive digenic association between *b5* and *e14* genes predicts that in populations where *e14* is low or absent, the loss of the *b* locus polymorphism becomes more likely, the conjunction of trigenic and monogenic (heterotic) effects suggest that, on the contrary, the selective advantage of *b* locus diversity is increased and, by consequence, *b* locus polymorphism more likely preserved in situations where *e* locus heterozygosity is low.

Compensatory aspects of Ig diversity could help explain the quite surprising observation that in the original Mediterranean range of the species, where total *b* locus heterozygosity approaches 90% (VAN DER LOO, FERRAND and SORIGUER 1991), *e* locus heterozygosity was found to be zero (*i.e.*, all of more than

600 rabbits from 30 localities from Southern France, Portugal, Spain and Azores, were homozygous *e15*; VAN DER LOO 1986, 1987; W. VAN DER LOO, N. FERRAND and M. MONNEROT, unpublished). By contrast, a recent study on 1340 feral rabbits from the Kerguelen archipelago revealed higher than average heterozygosities at the *e* locus ($H_T = 42\%$) while the *b* locus was fixed for the *b4* allele (BOUSSÈS 1992; W. VAN DER LOO, P. BOUSSÈS, CH. ARTHUR and J.-L. CHAPUIS, unpublished). By pooling the data on Kerguelen rabbits with those presented here, the associations between *b* locus heterozygosity and the *e15* allele becomes highly significant ($D_{bBE} = 0.0078$, $\chi^2 = 15$). Taken together these observations indicate that, in populations where most rabbits are homozygous *e15*—which is the situation prevailing in the original range of the species—the process responsible for the excess in *b* locus heterozygotes might be more effective than in the populations studied here where *e* locus heterozygosity was about 30%.

Comparisons with homozygote deficiency at mouse *MHC* loci: In a widely commented study on seminatural populations of mice by POTTS, MANNING and WAKELAND (1991) evidence was presented for *MHC* type-specific disassortative mating. The resulting deviations in genotypic frequencies of Hardy-Weinberg proportions are believed to play an essential role in maintaining allele diversity at *MHC* loci and were measured by the deficiency in homozygotes or by the fixation index [*i.e.*, the deficiency in heterozygotes (HEDRICK 1992)]. The striking similarities between the evolutionary patterns at *b* locus and *MHC* loci (VAN DER LOO and VERDOODT 1992) have led to the suggestion that both polymorphisms might be maintained by deterministic processes that are equally effective at the population genetic level. As shown in Table 10, the reported data seem in agreement with this hypothesis. The minimal estimates of *b* locus heterozygosities in samples from the Iberian range as previously reported by VAN DER LOO, FERRAND and SORIGUER (1991) indicate that homozygote deficiencies and heterozygous excess at the rabbit *b* locus can indeed be quite similar to those revealed by POTTS and coworkers for *MHC* haplotypes in mice.

Concluding remarks: For the *b* locus of the rabbit Ig L-chain constant region, the distribution of allele diversity in individuals and in populations as well as the patterns of interallelic divergence as revealed by DNA sequence comparison, differ notably from expectations for neutral polymorphisms but meet predictions of population genetic and evolutionary theory on allele diversity under overdominant-type selection (overdominant or frequency dependent). Overdominant selection offers a possible explanation for the homozygote deficiencies and the low frequency variances here reported. By increasing the allelic persist-

ence times, overdominance may have contributed to the exceptional divergence between alleles and can account for the high heterozygosity levels in the original range of the species. Positive selection can also explain why the rate of nucleotide substitutions was higher at amino acid replacement sites than at synonymous sites. The nonrandom distribution of allele diversity at the IgG H-chain loci might have to do with deterministic interactions between H-chain and (L-chain) *b* locus genotypes.

Questions about the nature of mechanism underlying the observed heterozygous excess can't be solved by means of analysis of population variance. The nonrandom correlations could be the outcome of viability selection or of some other determinism, as reported for *MHC* loci (POTTS MANNING and WAKELAND 1991) where allele specific dissortive mating is apparently anticipating the adaptive value of allele diversity (HOWARD 1991; VAN DER LOO and VERDOODT 1992). Analysis of population variance, as here presented may, however, help to define theoretical models in which the evolutionary patterns as revealed by DNA sequence comparisons are the outcome of nonrandom gene distribution as observed at the population level (see Introduction). The most relevant parameters in such models are the effective population size *N* and the selection coefficients *s*, which are also the most elusive. The magnitude of the heterozygous excess here observed may help to design experiments from which reliable estimates of the (apparent) selection force can be derived. Such estimates may allow to answer the question whether the product of the selection force and the effective population size *Ns* can, in models proposed in the literature (TAKAHATA 1990, TAKAHATA and NEI 1990), satisfy available data on evolution rates, population diversity and coalescence times of *b* locus alleles.

Studies on the rabbit *Ig* polymorphisms may by this provide an *a contrario* argument in support of the Neutral Theory by verifying that deterministic effects on allele correlations within individuals do indeed impose modes of population genetic and evolutionary variation that can be distinguished from expectation under random drift on neutral alleles, an implicit hypothesis in most arguments in favor of the selective neutrality of observed genetic variance. To immunogeneticists these studies may reveal the importance of the *Ig* allotypes, which were often treated as selectively neutral genetic "markers." As parasites tend to manipulate the host's immune response by interacting with the antibodies (HAMERS, VAN DER LOO and DE BAETSELIER 1986; VAN DER LOO 1987), it should not be surprising that diversity of the constant regions of antibodies became part of a (host) defense strategy (*cf.* HALDANE 1949; HAMILTON, AXELROD and TANESE 1990; DAMIAN 1987), but the mode and the intensity

of the diversity enhancing mechanisms as inferred from the gene correlations, may be interesting. It appears that at least for some loci of the antibody constant region, patterns of polymorphism and divergence are in every respect similar to those described for human and mouse *MHC* loci, at both the evolutionary and population genetic level. The observations reported here furthermore indicate that the fact that *Ig* molecules are encoded by different loci, can create situations of particular interest for studies on multilocus interaction.

BARRY RICHARDSON, JOHN ROSS, MARIJKE, DREES-WALLAGE, CHRISTIAN ARTHUR, PETER GARSON and PHILIP KERREMANS have contributed to the collection of the serum samples. I was privileged to dispose of a large panel of well characterized antisera which is the spin off of 25 years of research and animal breeding by RAYMOND HAMERS and CECILE HAMERS-CASTERMANS. I am furthermore indebted to DOMIEN ROGGEN for interesting discussions and particularly to PAUL SUNNICK, who encouraged me to publish these data and who corrected an earlier version of the manuscript. The author is senior research associate of the Belgian National Science Foundation (N.F.W.O.-F.N.R.S.)

LITERATURE CITED

- AVISE, J. C., M. H. SMITH and R. K. SELANDER, 1979 Biochemical polymorphism and systematics in the genus *Peromyscus*. VII. Geographic differentiation in members of the *Truei* and *Maniculatus* species groups. *J. Mammol.* **60**: 177-192.
- BARKER, J. F. F., P. D. EAST and B. S. WEIR, 1986 Temporal and microgeographic variation in allozyme frequencies in a natural population of *Drosophila buzzatii*. *Genetics* **112**: 577-611.
- BOUSSÉS, P., 1992 Biologie de population d'un vertébré phytophage introduit, le Lapin de Garenne (*Oryctolagus cuniculus*), dans les îles subantarctiques de Kerguelen. Thèse de Doctorat 726, Université de Rennes, Rennes, France.
- CAVALLI-SFORZA, L. L., 1966 Population structure in human evolution. *Proc. R. Soc. Lond. B* **164**: 362-379.
- CHAKRABORTY, R., 1974 A note on Nei's measure of gene diversity in a substructured population. *Humangenetik* **21**: 85-88.
- CLARKE, B., 1979 The evolution of genetic diversity. *P. Roy. Soc. Lond. B.* **205**: 453-474.
- COCKERHAM, C. C., 1973 Analyzes of gene frequencies. *Genetics* **74**: 679-700.
- CROW, J. F., and M. KIMURA, 1970 *An Introduction to Population Genetics Theory*. Harper & Row, New York.
- DALY, J. C., 1979 The ecological genetics of the European wild rabbit (*Oryctolagus cuniculus*) in Australia. Ph.D. Thesis, Australian National University, Canberra, Australia.
- DAMIAN, R. T., 1987 Molecular mimicry revisited. *Parasitol. Today* **3**: 263-266.
- DRAY, S., 1962 Effect of maternal isoantibodies on the quantitative expression of two allelic genes controlling g-globulin allotypic specificities. *Nature* **195**: 677-680.
- DRAY, S., 1972 Allotype suppression, pp. 87-102 in *Ontogeny of Acquired Immunity*, edited by R. PORTER and J. KNIGHT. Elsevier, Amsterdam.
- EMORINE, L., J. A. SOGN, F. D. TRING, T. J. KINDT and E. E. MAX, 1984 A genomic gene encoding the *b5* rabbit immunoglobulin kappa constant region. *Proc. Natl. Acad. Sci. USA* **81**: 1789-1793.
- EWENS, W. J., and M. W. FELDMANN, 1976 The theoretical assessment of selective neutrality, in *Population Genetics and Ecology*, edited by S. KARLIN and E. NEVO. Academic Press, New York.

- FENNER, F., 1953 Changes in mortality-rate due to myxomatosis in Australian wild rabbit. *Nature* **172**: 228.
- FENNER, F., and F. N. RATCLIFFE, 1965 *Myxomatosis*. Cambridge University Press, New York.
- FIGUEROA, F., E. GÜNTHER and J. KLEIN, 1988 MHC polymorphism pre-dating speciation. *Nature* **335**: 265-267.
- GOJOBORI, T., 1982 Means and variances of heterozygosity and protein function, pp. 137-150 in *Molecular Evolution, Protein Polymorphism and the Neutral Theory*, edited by M. KIMURA. Japan Scientific Societies Press, Tokyo.
- GRUBB, R., 1970 *The Genetic Markers of Human Immunoglobulins*. Springer Verlag, Berlin.
- HALDANE, J. B. S., 1949 Disease and evolution. *Ric. Sci.*, **19**: 68.
- HAMERS, R., 1987 Allotropy of immunoglobulins. pp. 65-77, in *The Rabbit in Contemporary Immunological Research*, edited by S. DUBISKI. John Wiley & Sons, New York.
- HAMERS, R., W. VAN DER LOO and P. DE BAETSELIER, 1986 Structure and function of immunoglobulins, the symbiotic interplay between parasites and host antibodies, pp. 324-345 in *The Ruminant Immune System in Health and Disease*, edited by I. MORRISON. Cambridge University Press, Cambridge.
- HAMILTON, W. D., R. AXELROD and R. TANESE, 1990 Sexual reproduction as an adaptation to resist parasites (a review). *Proc. Natl. Acad. Sci. USA* **87**: 3566-3573.
- HARTL, D. L., 1981 *A Primer of Population Genetics*. Sinauer Assoc., Sunderland, Mass.
- HEDRICK, P. W., and G. THOMPSON, 1983 Evidence for balancing selection at HLA. *Genetics* **104**: 449-456.
- HEDRICK, P. W., 1990 Selection at HLA: possible explanations for deficiency of homozygotes. *Hum. Hered.* **40**: 213-220.
- HEDRICK, P. W., 1992 Female choice and variation in the major histocompatibility complex. *Genetics* **132**: 575-581.
- HERD, Z. L., and J. W. E. EDMONDS, 1977 Population genetics of Aa and Ab immunoglobulin allotypes in wild rabbits of South-eastern Australia. I. Allotype frequencies. *J. Immunogenet.* **4**: 315-324.
- HERD, Z. L., and J. W. E. EDMONDS, 1981 Population genetics of Aa and Ab immunoglobulin allotypes in Australian wild rabbit *Oryctolagus cuniculus*. *Fed. Proc.*, **39**: Abstract 989.
- HERZENBERG, L. A., and L. A. HERZENBERG, 1978 Mouse immunoglobulin allotypes: description and specific methodology, Ch. 12 in *Handbook of Experimental Immunology*, Ed. 3, edited by D. M. WEIR. Blackwell Scientific Publications, Oxford.
- HOLLAND, C. W., 1923 Rabbits and their introduction into Australia. *Queensl. Nat.* **4**: 7-34.
- HOWARD, J. C., 1991 Disease and evolution. *Nature* **352**: 565-567.
- HUGHES, A., and M. NEI, 1988 Patterns of nucleotide substitution at major histocompatibility complex class loci reveals overdominant selection. *Nature* **335**: 167-170.
- HUGHES, A., and M. NEI, 1989 Nucleotide substitution at major histocompatibility complex class II loci: evidence for overdominant selection. *Proc. Natl. Acad. Sci. USA* **86**: 958-962.
- KELUS, A., and P. GELL, 1967 Immunoglobulin allotypes of experimental animals. *Prog. Allergy* **11**: 141-156.
- KERREMANS, PH., 1982 Studie van de Interlokale Varianties in Pecking Orde van de Uitdrukking van Immunoglobuline Allotypen. Licentiaats Thesis, Vrije Universiteit Brussels, Brussels, Belgium.
- KIDDLE, M., 1961 *Men of Yesterday. A Social History of the Western District of Victoria*. Melbourne University Press.
- KIMURA, M., 1968 Evolution rate at the molecular level. *Nature* **217**: 624-626.
- KING, J. L., and T. H. JUKES, 1969 Non-Darwinian evolution: random fixation of selectively neutral mutations. *Science* **164**: 788-798.
- KLEIN, J., 1986 *Natural History of the Major Histocompatibility Complex*. Wiley, New York.
- LEWONTIN, R. C., 1974 *The Genetic Basis of Molecular Change*. Columbia University Press, New York.
- LEWONTIN, R. C., L. R. GINZBURG and S. TULJAPURKAR, 1987 Heterosis as an explanation for large amounts of genic polymorphism. *Genetics* **88**: 149-170.
- LEWONTIN, R. C., and J. KRAKAUER, 1973 Distribution of gene frequencies as a test of the theory of the selective neutrality of polymorphisms. *Genetics* **74**: 175-195.
- LI, W.-H., 1978 Maintenance of genetic variability under the joint effect of mutation, selection and random drift. *Genetics* **90**: 349-382.
- LOYDS, H. G., 1981 Biological observations on post-myxomatosis wild rabbit populations in Britain, 1955-79, pp. 623-628 in *Proceedings World Lagomorph Conference Guelph, 1979*, edited by K. MYERS and C. D. MCINNES. University of Guelph Publ., Guelph, Ontario, Canada.
- LUMMUS, Z., J. J. CEBRA, and R. MAGE, 1967 Correspondence of the relative cellular distribution and serum concentration of allelic allotypic markers in normal and allotype suppressed heterozygous rabbits. *J. Immunol.* **99**: 737-740.
- MAGE, R. G., 1986 Rabbit immunoglobulin allotypes, pp. 164-190 in *Handbook of Experimental Immunology*, Vol. 3, edited by D. M. WEIR, L. A. HERZENBERG, C. C. BLACKWELL and L. A. HERZENBERG. Blackwell Scientific Publications, Oxford.
- MAGE, R. G., 1987 Molecular biology of rabbit immunoglobulin and T-cell receptor genes, pp. 106-133 in *The Rabbit in Contemporary Immunological Research*, edited by S. DUBISKI. John Wiley & Sons, New York.
- MARUYAMA, T., and M. NEI, 1981 Genetic variability maintained by mutation and overdominant selection in finite populations. *Genetics* **98**: 441-459.
- MATTHYSSENS, G., B. BORREMANS, R. HAMERS and W. VAN DER LOO, 1985 Genomic evidence for the truly allelic nature of the complex b-locus allotypes of the rabbit immunoglobulin light chain, pp. 123-126 in *Protides of the Biological Fluids, Proceedings of the 33th Colloquium 1984*, edited by H. PEETERS. Pergamon Press, Oxford.
- MYERS, K., I. D. MARSHALL and F. FENNER, 1954 Studies in the epidemiology of infectious myxomatosis of rabbits. III. Observations on two succeeding epizootics in Australian wild rabbits on the Riverine plain of south-eastern Australia, 1951-1953. *J. Hyg.* **52**: 337-346.
- NADEAU, J. H., J. BRITTON-DAVIDSON, F. BONHOMME and L. THALER, 1988 H-2 polymorphisms are more uniformly distributed than allozyme polymorphisms in natural populations of house mice. *Genetics* **118**: 131-140.
- LOUDIN, J., 1960 Allotropy of rabbit serum proteins. I. Immunochemical analysis leading to the individualization of seven main allotypes. *J. Exp. Med.* **112**: 107-124.
- POTTS, W., C. J. MANNING and E. W. WAKELAND, 1991 Mating patterns in seminatural populations of mice influenced by MHC genotype. *Nature* **352**: 619-621.
- RICHARDSON, B. J., 1981 The genetic structure of rabbit populations, pp. 37-52 in *Proceedings World Lagomorph Conference Guelph, 1979*, edited by K. MYERS and C. D. MCINNES, University of Guelph Publ., Guelph, Ontario, Canada.
- RICHARDSON, B. J., P. M. ROGERS and G. M. HEWITT, 1980 Ecological genetics of the wild rabbit in Australia. II. Protein variation in British, French and Australian rabbits and the geographical distribution of the variation in Australia. *Aust. J. Biol. Sci.* **33**: 371-383.
- RITCHIE, K. A., R. L. BRINSTER and U. STORB, 1984 Allelic exclusion and control of endogenous immunoglobulin gene rearrangement in K transgenic mice. *Nature* **312**: 517-520.
- REISFELD, R. A., S. DRAY and A. NISONOFF, 1965 Differences in amino acid composition of rabbit IgG-immunoglobulin light

- chains controlled by allelic genes. *Immunochemistry* **2**: 155–167.
- REYNOLDS, J., B. S. WEIR and C. C. COCKERHAM, 1983 Estimation of coancestry coefficients: basis for a short term genetic distance. *Genetics* **105**: 767–779.
- ROBERTSON, A., 1975 Remarks on the Lewontin-Krakauer test. *Genetics* **80**: 396.
- ROSS, T. J., and A. M. TITTENSOR, 1981 Myxomatosis in selected rabbit populations in South-east England and Wales, 1971–1978, pp. 830–833 in *Proceedings World Lagomorph Conference Guelph, Ontario, 1979*, edited by MYERS, K. and C. D. MAC-INNES, University of Guelph Publ., Guelph, Ontario, Canada.
- ROSS, J. J., and A. M. TITTENSOR, 1986 Influence of myxomatosis in regulating rabbit numbers. *Mammal. Rev.* **16**: 163–168.
- SELANDER, R. K., and D. W. KAUFMAN, 1975 Genetic structure of populations of the brown snail (*Helix aspersa*). I. Microgeographic variation. *Evolution* **29**: 385–401.
- SINGH, R. S., and L. R. RHOMBERG, 1987 A comprehensive study of genetic variation in natural populations in *Drosophila melanogaster*. II. Estimates of heterozygosity and patterns of geographic differentiation. *Genetics* **117**: 255–271.
- SOUTHERN, H. N., 1940 The ecology and population dynamics of the wild rabbit, *Oryctolagus cuniculus*. *Ann. Appl. Biol.* **27**: 509–526.
- TAKAHATA, N., 1990 A simple genealogical structure of strongly balanced allelic lines and trans-species evolution of polymorphism. *Proc. Natl. Acad. Sci. USA* **87**: 2419–2423.
- TAKAHATA, N., and M. NEI, 1990 Allelic genealogy under overdominance and frequency-dependent selection and polymorphism of the major histocompatibility complex loci. *Genetics* **124**: 967–978.
- TAKAHATA, N., Y. SATTA and J. KLEIN, 1992 Polymorphism and balancing selection at major histocompatibility complex loci. *Genetics* **130**: 925–938.
- VAN DER LOO, W., 1986 Geographical immunogenetics of European rabbit. *Mammal. Rev.* **16**: 199.
- VAN DER LOO, W., 1987 Studies on the adaptive significance of the immunoglobulin alleles (Ig allotypes) in wild rabbit, pp. 164–190 in *The Rabbit in Contemporary Immunological Research*, edited by S. DUBISKI. John Wiley & Sons, New York.
- VAN DER LOO, W., N. FERRAND and R. SORIGUER, 1991 Estimation of gene diversity at the *b*-locus of the constant region of the immunoglobulin light chain in natural populations of European rabbit (*Oryctolagus cuniculus*) in Portugal, Andalusia and on the Azorean Islands. *Genetics* **127**: 789–799.
- VAN DER LOO, W., and VERDOODT, 1992 Patterns of interallelic divergence at the rabbit *b*-locus of the immunoglobulin light chain constant region are in agreement with population genetic evidence for overdominant selection. *Genetics* **132**: 1105–1117.
- VAN DER LOO, W., C. P. ARTHUR, B. RICHARDSON, M. WALLAGE and R. HAMERS, 1987 Nonrandom allele associations between unlinked protein loci: are the allotypes of the immunoglobulin constant regions adaptive? *Proc. Natl. Acad. Sci. USA* **84**: 3075–3079.
- WATTERSON, G. A., 1978 The homozygosity test of neutrality. *Genetics* **88**: 405–417.
- WEIR, S., 1990 *Genetic Data Analysis*. Sinauer Assoc., Sunderland, Mass.
- WEIR, B. S., and C. C. COCKERHAM, 1984 Estimation of F-statistics for the analysis of population structure. *Evolution* **38**: 1358–1370.
- WEIR, B. S., and C. C. COCKERHAM, 1989 Complete characterisation of disequilibrium at two loci, pp. 86–110 in *Mathematical Evolutionary Theory*, edited by M. E. FELDMAN. Princeton University Press, Princeton, N.J.
- WRIGHT, S., 1951 The genetic structure of populations. *Ann. Eugen.* **15**: 323–354.
- WRIGHT, S., 1978 *Evolution and Genetics of Populations*, Vol. 4. University of Chicago Press, Chicago.
- ZEUNER, F. E., 1963 *A History of Domesticated Animals*. Hutchison, London.

Communicating editor: B. S. WEIR

APPENDIX

Numbers of combined L- and H-chain genotypes observed per locality are given in Table 11.

TABLE 11
Numbers of combined L- and H-chain genotypes observed per locality

	Genotype <i>bbbaaadDeE</i> 459123 ^a	Locality																
		BB	BW	CH	CN	MO	QU	UN	UR	GC	DO	GL	HI	PO	NH	WE	CL	TOT
1	2002002002	-	-	1	-	-	-	-	-	2	-	-	-	-	-	-	3	6
2	2002001111	-	-	-	1	-	-	-	-	10	-	-	-	-	-	-	-	11
3	2002001102	1	-	-	-	-	1	1	2	11	2	-	-	-	-	-	3	21
4	2002000220	-	-	1	2	-	-	-	-	12	-	-	-	1	-	-	-	16
5	2002000211	-	-	6	2	1	-	1	2	30	2	1	7	1	-	-	-	53
6	2002000202	-	1	-	-	2	-	-	10	35	10	1	9	-	1	1	6	76
7	2001101111	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	1	2
8	2001101102	-	-	-	-	-	-	-	2	7	-	-	-	-	-	-	2	11
9	2001100220	1	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	2
10	2001100211	-	1	-	4	-	-	-	5	12	2	-	-	-	3	-	3	30
11	2001100202	-	-	-	3	-	-	-	3	18	-	1	-	1	1	-	1	28
12	2001012002	3	-	-	-	-	-	-	2	14	2	-	-	-	-	-	1	22
13	2001011111	2	1	-	1	-	-	-	1	15	3	-	7	1	-	-	-	31
14	2001011102	3	2	6	3	-	-	1	7	41	20	1	16	-	4	-	5	109
15	2001010211	-	-	7	7	1	-	1	-	14	-	-	3	1	1	-	1	36
16	2001010202	-	1	4	5	-	1	2	-	18	6	1	11	1	5	-	3	58
17	2000202002	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	1
18	2000200220	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2	2
19	2000200211	-	-	-	1	-	-	-	4	2	-	-	-	-	3	-	1	11
20	2000200202	-	-	-	-	-	-	-	-	2	-	-	-	-	-	-	-	2
21	2000112002	-	-	-	-	-	-	-	2	-	-	-	-	-	-	-	-	2
22	2000111111	2	1	-	-	-	-	-	-	1	1	-	-	-	1	1	-	7
23	2000111102	1	2	-	1	-	-	-	1	8	-	-	-	-	3	-	1	17
24	2000110220	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	1
25	2000110211	-	1	-	-	-	-	1	-	-	-	-	-	-	2	-	5	9
26	2000110202	-	-	-	4	1	-	-	-	5	-	-	-	-	1	1	4	16
27	2000022002	2	2	-	-	-	-	-	-	11	17	2	5	-	6	3	1	49
28	2000021111	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	1	2
29	2000021102	2	1	1	-	-	-	-	-	12	3	1	11	-	8	-	2	41
30	2000020220	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	1
31	2000020211	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	2	3
32	2000020202	-	-	7	4	-	1	1	-	13	2	-	6	-	8	-	2	44
33	2000002002	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	1
34	1102002002	-	-	-	-	-	1	-	-	5	-	-	-	-	-	-	-	6
35	1102001111	-	-	3	-	-	-	-	-	13	-	-	-	-	-	-	-	16
36	1102001102	-	-	-	2	-	-	-	-	17	-	-	-	-	-	-	3	22
37	1102000220	-	-	2	-	-	1	-	-	11	-	1	-	1	-	-	-	16
38	1102000211	-	-	4	4	4	-	1	1	16	1	3	-	2	-	2	1	39
39	1102000202	1	1	2	-	1	-	1	7	18	3	1	-	2	2	1	6	46
40	1101102002	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	1
41	1101101111	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	1	2
42	1101101102	-	-	-	-	-	-	-	2	3	-	-	-	-	-	-	-	5
43	1101100220	-	-	-	-	-	-	-	-	2	-	-	-	2	-	2	-	6
44	1101100211	-	3	1	2	-	-	1	1	9	1	-	-	-	-	1	5	24
45	1101100202	1	-	-	1	-	-	-	4	6	-	-	-	-	2	1	1	16
46	1101012002	-	1	-	-	-	-	-	-	11	-	-	-	-	-	-	-	12
47	1101011111	-	-	1	1	-	-	-	-	13	3	1	-	-	-	1	1	21
48	1101011102	1	3	2	1	-	-	2	1	41	4	4	-	-	4	-	7	70
49	1101010220	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	1
50	1101010211	-	-	3	2	-	-	1	-	18	-	1	-	2	2	-	3	32
51	1101010202	-	2	6	5	-	-	2	-	17	-	1	-	-	10	3	7	53
52	1100201111	-	-	-	-	-	-	-	1	-	-	-	-	-	-	1	-	2
53	1100201102	-	-	-	-	-	-	-	1	-	-	-	-	-	-	1	-	2
54	1100200220	-	1	-	-	-	-	-	-	-	-	-	-	1	1	-	1	4
55	1100200211	-	2	-	1	-	-	-	2	1	-	-	-	-	3	2	1	12
56	1100200202	-	-	-	1	-	-	-	-	2	-	-	-	-	-	1	-	4
57	1100112002	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	1
58	1100111111	-	1	-	-	1	-	-	1	-	1	-	-	-	-	-	1	5
59	1100111102	-	2	-	-	-	-	-	3	3	-	-	-	-	5	-	-	13

TABLE 11—Continued

	Genotype <i>bbbaadDeE</i> 459123 ^a	Locality																TOT
		BB	BW	CH	CN	MO	QU	UN	UR	GC	DO	GL	HI	PO	NH	WE	CL	
60	1100110220	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	4	5
61	1100110211	-	2	-	-	-	-	-	-	-	-	-	-	-	3	-	3	8
62	1100110202	1	-	-	2	-	-	-	-	3	-	-	-	-	4	1	1	12
63	1100022002	1	2	-	-	-	-	-	-	12	5	-	-	1	8	1	-	30
64	1100021111	-	-	-	-	-	-	-	-	-	-	-	-	-	2	-	2	4
65	1100021102	-	1	2	-	-	1	-	-	5	1	-	-	1	8	2	-	21
66	1100020211	-	-	-	-	-	-	-	-	-	-	-	-	-	4	-	5	9
67	1100020202	-	1	7	6	-	1	-	-	6	-	-	-	2	5	1	4	33
68	1012001102	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	1	2
69	1012000220	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	1
70	1012000211	-	-	-	-	-	-	-	-	1	-	-	-	2	-	-	-	3
71	1012000202	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	1
72	1011101111	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	1	2
73	1011100220	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	1
74	1011100211	-	-	-	-	-	-	-	-	1	-	-	-	1	-	-	2	4
75	1011100202	-	-	-	-	1	-	-	1	-	-	-	-	1	-	-	-	3
76	1011011102	-	-	-	-	-	-	-	1	4	-	-	-	1	-	-	2	8
77	1011010211	-	-	-	-	-	-	-	-	-	-	-	-	2	-	-	1	3
78	1011010202	-	-	-	-	-	-	-	-	4	-	-	-	-	-	-	2	6
79	1010200220	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	1	2
80	1010200211	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	1
81	1010200202	-	-	-	1	-	-	-	-	1	-	-	-	-	-	-	-	2
82	1010112002	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	1
83	1010111102	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	1	2
84	1010110220	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	1
85	1010110211	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	1
86	1010022002	-	-	-	-	-	-	-	-	2	-	-	-	-	-	-	-	2
87	1010021102	-	-	-	-	-	-	-	-	2	-	-	-	2	-	-	1	5
88	1010020211	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	1
89	1010020202	-	-	3	-	-	-	-	-	-	-	-	-	2	-	-	2	7
90	0202001111	-	-	-	2	-	-	-	-	1	-	-	-	-	-	-	-	3
91	0202001102	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	2	3
92	0202000220	-	-	-	1	-	-	-	-	1	-	-	-	2	-	1	-	5
93	0202000211	1	-	3	1	-	-	-	-	4	-	-	-	2	-	-	-	11
94	0202000202	-	-	-	-	-	-	-	-	1	-	1	-	-	-	1	-	3
95	0201101111	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	1
96	0201101102	-	-	-	-	-	-	-	1	1	-	-	-	-	-	-	-	2
97	0201100220	-	-	-	-	-	-	-	-	-	-	-	-	4	-	2	-	6
98	0201100211	-	1	-	-	1	-	-	-	3	-	1	-	3	1	1	1	12
99	0201100202	-	-	-	-	-	-	-	-	-	-	-	-	2	-	1	1	4
100	0201012002	-	-	-	-	-	-	-	1	1	-	-	-	-	-	-	-	2
101	0201011111	-	-	1	-	-	-	-	-	2	-	-	-	2	-	2	-	7
102	0201011102	-	1	-	-	-	-	-	-	3	-	-	-	-	1	3	-	8
103	0201010211	-	-	4	-	-	-	1	-	2	-	-	-	1	-	-	-	8
104	0201010202	-	-	3	-	-	-	-	-	1	-	-	-	1	-	-	3	8
105	0200201111	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	1
106	0200200220	-	-	-	-	-	-	1	-	-	-	-	-	1	1	2	-	5
107	0200200211	-	1	-	-	-	-	-	-	-	-	-	-	1	-	-	-	2
108	0200111111	-	1	-	-	-	-	-	-	-	-	-	-	1	-	1	-	3
109	0200111102	-	-	1	-	-	-	-	-	1	-	-	-	-	-	-	-	2
110	0200110211	-	-	-	-	-	-	-	-	-	-	-	-	2	1	2	1	6
111	0200110202	-	-	-	-	-	-	-	-	-	-	-	-	-	3	1	-	4
112	0200022002	-	-	-	-	-	-	-	-	2	-	-	-	-	-	2	-	4
113	0200021102	-	-	-	-	-	-	-	-	-	-	-	-	4	2	2	-	8
114	0200020220	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	1
115	0200020202	-	-	2	-	-	-	2	-	1	-	-	-	1	3	1	-	10
116	0112002002	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	1
117	0112001102	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	2	3
118	0112000220	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	1

TABLE 11—Continued

	Genotype <i>bbbaaadDeE</i> <i>459123^a</i>	Locality																TOT
		BB	BW	CH	CN	MO	QU	UN	UR	GC	DO	GL	HI	PO	NH	WE	CL	
119	0112000211	-	-	-	-	-	-	-	-	-	-	-	-	5	-	-	-	5
120	0112000202	-	-	-	-	2	-	-	-	1	-	-	-	1	-	-	-	4
121	0111100220	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	1
122	0111100211	-	-	-	-	-	-	-	-	2	-	-	-	5	-	-	1	8
123	0111100202	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	1
124	0111012002	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	1
125	0111011102	-	-	-	-	-	-	-	-	1	-	-	-	1	-	-	-	2
126	0111010211	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	1
127	0111010202	-	-	-	-	-	-	-	-	-	-	-	-	9	-	-	1	10
128	0110201111	-	-	-	-	-	-	-	-	-	-	-	-	2	-	-	-	2
129	0110200211	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	1	2
130	0110200202	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	1
131	0110111111	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	1
132	0110110220	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	1
133	0110110211	-	-	-	-	-	-	-	-	-	-	-	-	3	-	-	1	4
134	0110022002	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	1
135	0110021102	-	-	-	-	-	-	-	-	-	-	-	-	2	-	-	-	2
136	0110020211	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	1
137	0110020202	-	-	-	-	-	-	-	-	2	-	-	-	4	-	-	-	6
138	0022000202	-	-	-	-	-	-	-	-	-	-	-	-	3	-	-	1	4
139	0021100211	-	-	-	-	-	-	-	-	-	-	-	-	3	-	-	-	3
140	0021011111	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	1
141	0021011102	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	1
142	0021010202	-	-	-	-	-	-	-	-	-	-	-	-	2	-	-	-	2
143	0020200202	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	1
144	0020111111	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	1
145	0020111102	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	1
146	0020110211	-	-	-	-	-	-	-	-	-	-	-	-	2	-	-	-	2
147	0020021102	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	1
148	0020020202	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	1
		23	39	83	71	15	7	21	77	575	89	22	75	117	125	53	147	1539

The presented data list allows the calculus of all parameters presented in this paper as well as a complete analysis of the linkage disequilibria, which will be discussed elsewhere. The most frequent are rabbits with genotype number 14 "2001011102" which reads *b4/b4; a1/a3; d11/d12; e15/e15*.

^a Allele is at homozygous state "2," at heterozygous state "1" or is absent "0." Genotype names reflect the sequence of states of alleles *b4, b5, b9, a1, a2, a3, d11, d12, e14* and *e15*, respectively.