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Compensatory Aspects of Allele Diversity at Immunoglobulin Loci: Gene Correlations in Rabbit Populations Devoid of Light Chain Diversity (*Oryctolagus cuniculus* L.; Kerguelen Islands)

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ABSTRACT

Is there a selective advantage of increased diversity at one immunoglobulin locus when diversity at another locus is low? A previous paper demonstrated excess heterozygosity at the rabbit light chain *b* locus when heterozygosity was low at the heavy chain constant region *e* locus. Here we consider the reverse situation by analyzing allele distributions at heavy chain loci in populations fixed for the light chain *b* locus. We analyzed the *a* locus that encodes the predominantly expressed heavy chain variable region, and the *d* and *e* loci that control different parts of the Ig gamma class constant region. While there was excess heterozygosity, genetic differentiation between localities was extensive and was most pronounced for females. This was in marked contrast with observations in areas where *b*-locus diversity was important and confirms a negative correlation between *e*- and *b*-locus heterozygosity. Trigenic disequilibria corresponded to a significant negative correlation between *e*- and *a*-locus heterozygosity due mainly to strong variation *among* localities within the context of pronounced (digenic) linkage disequilibria. Although substantial, the average increase in *a*/*e*-locus single heterozygosity implemented by higher order disequilibria *within* localities was not significant.

MOST genetic variation can be explained by neutral mutations and random drift (KIMURA 1968, 1987). There is, however, growing evidence that the extensive polymorphisms observed at loci involved in immune response are the outcome of deterministic processes (diversity enhancing selection). The detailed study of the patterns of diversity at histocompatibility loci (*Mhc*) provide strong evidence for overdominance-type selection at these loci (for a recent synopsis see KLEIN and O'HUIGIN 1995). It appears that histocompatibility loci offer a rare, although particular, case where effects of selection on molecular evolutionary modes can be studied.

Extensive genetic variation is not found only for *Mhc* loci. The constant (*sic*) parts of the immunoglobulin (Ig) molecule (*i.e.*, the effector part of the antibody) are also controlled by loci that can be highly polymorphic (*cf.* HERZENBERG and HERZENBERG 1978; MAGE 1986). Patterns of gene diversity at *Ig* loci might be of particular interest to evolutionary genetics: *Ig* genes do not exist as such in the germline, but are generated during ontogeny by recombination of more or less distant segments that are scattered along the chromosomes (TONEGAWA 1983; *reviewed in* LEWIN 1995). The multi-locus control of antibody synthesis provides an investigative instrument of a key hypothesis in evolution theory, which claims that context and interaction between genes at different loci are essential issues in adaptive variation (LEWONTIN 1974). For reasons related to the particular history and behavior of the European rabbit (*Oryctolagus cuniculus* L.) a study of the evolutionary genetics of the rabbit *Ig* loci is of specific interest (*cf.* VAN DER LOO 1987). In this large "small-mammal" species, well characterized serologic markers of allelic variation exist for loci determining components of the antibody heavy chain (H chain) and light chain (L chain), respectively (reviewed in MAGE 1986). For the H chain, allelic markers are available for both the *variable* region (VH) and the constant region of the major antibody class, IgG. This situation is exceptional, because allelic diversity is generally not observed at the antibody variable region (see below). The close linkage of loci encoding, respectively, the variable and constant parts of the H chain and the absence of linkage between H and L chain loci should, in effect, provide a suitable case model for studies of multi-locus systems exposed to selection.

The patterns of allele diversity at the rabbit Ig L chain *constant* region CK1 (*IgCK1* or *b* locus), were found to be very similar to those reported for *Mhc* loci: (1) with seven alleles sequenced so far, allelic variation was observed at 48 of the 102–104 amino acids composing the CK1 domain (VAN DER LOO *et al.* 1995); (2) at the DNA level, a significant bias toward amino acid altering substitutions was observed (for details and references

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see VAN DER LOO and VERDOODT 1992); (3) in natural populations, expected heterozygosity levels were consistently high (40–87%) (HERD and EDMONDS 1977; CAZENAVE *et al.* 1987; VAN DER LOO *et al.* 1991) while a 10% heterozygosity excess was documented (VAN DER LOO 1993); (4) in spite of the fact that L chain and H chain loci are on different chromosomes, *b* locus alleles showed significant nonrandom associations with alleles of H chain loci (VAN DER LOO *et al.* 1987; VAN DER LOO 1993); (5) subspecies showing 4% mitochondrial DNA divergence share alleleic lineages, implying allele coalescence times of $>10^6$ per year (VAN DER LOO *et al.* 1991, 1995). Such patterns constitute convincing evidence for overdominance-type selection (*cf.* HUGHES and NEI 1989; TAKAHATA *et al.* 1992).

In marked contrast with the extraordinary diversity at the L chain constant region, the H chain constant region of the rabbit antibody has been described as "*unique in its lack of genetic diversity*" (HAMERS 1987). Unlike other mammals, in rabbit there are no subclasses of IgG. All rabbit IgG molecules share the same constant region (CG) that is encoded by a single gene segment (*IgCG*). Allelic variation at this locus is very limited when compared to the polymorphisms reported for the different *IgCG* genes in rodents and primates. Furthermore, although the rabbit, like other vertebrates, disposes of more than one hundred functional *VH* gene segments, 70–90% of rabbit antibody heavy chains are, at the species level, derived from the same *VH* gene (KNIGHT and BECKER 1990). In other species, a large number of *VH* genes contribute to the diversity of the variable region while at the same time, some four distinct polymorphic constant region genes are involved in the synthesis of the major antibody class (IgG represents ~95% of circulating antibodies). However in rabbit, the vast majority of IgG heavy chains are derived by assembling the same *VH* and *CG* gene segments (for more details on the *VH* gene usage in rabbit see FRIEDMAN *et al.* 1994). It is therefore interesting that the preferentially expressed *VH* gene (*VHa*) displays an unusual degree of allelic variation, which corresponds to the *a* locus allotypes (BECKER and KNIGHT 1990; SHORT *et al.* 1991). As is the case for the *b* locus, the *a* locus alleles differ by multiple amino acid substitutions and 13 alleles have been described up to now (HAOUAS *et al.* 1989). These observations underlie the hypothesis that the extensive allelic variability at the rabbit *a* and *b* loci is adaptive and somehow compensates for the restrained diversity at the *Ig* H chain components.

In the aboriginal range of the species, wild populations show much larger numbers of alleles at *IgVHa* and at *IgCK1* loci (*a* locus and *b* locus, respectively) when compared to populations of the more "recent" distribution range [the aboriginal region of the species being limited to Iberian peninsula and Southern France; reviewed in ROGERS ARTHUR and SORIGUER (1994) and in CALLOU (1995)]. Such reduction in population diversity was also documented for other nuclear and mitochondrial markers (FERRAND 1995; HARDY *et al.* 1995). The *e*-locus polymorphism of the IgG constant region (*IgCG*), constitutes a notable exception. For populations of the recent distribution area, heterozygosity at the *e* locus was always higher than 15% ($H_p = 0.32 \pm 0.09$; VAN DER LOO 1993). However, all populations of the aboriginal area were homozygous (fixed) for the *e15* allele, as were most populations of other leporid species (VAN DER LOO 1986, 1987; C. P. ARTHUR, E. CASTIEN, N. FERRAND, M. S. SANCHEZ, M. MONNEROT, R. SORIGUER, and W. VAN DER LOO, unpublished results). This led to the suggestion that the *e*-locus polymorphism, which depends upon a single transitional nucleotide substitution, evolved within the founder population of the rabbits of the recent range (VAN DER LOO, unpublished results), a hypothesis that is also supported by the pronounced linkage disequilibria between *e* locus alleles and alleles of the closely linked *a* locus (VAN DER LOO and ARTHUR 1987; *Note:* the presence of the *e14* serotype in other species can be explained by convergence).

Owing to the fact that H chain and L chain loci are on different chromosomes, the observation of highly significant nonrandom associations (linkage disequilibrium) between *e* locus and *b* locus alleles both *between* and *within* populations was regarded as evidence for epistatic interactions between H and L chain constant regions (VAN DER LOO *et al.* 1987). More recently, trigenic disequilibria were reported between *b* and *e* loci. The observed associations could be explained by postulating that overdominance-type selection at the L chain *b* locus was more effective in rabbits that were homozygous at the H chain *e* locus (*compensatory* or *nonadditive* overdominance, see VAN DER LOO 1993). In the context of these apparent two-locus interactions, we have analyzed the distribution of gene diversity at the H chain loci in populations that were devoid of *b*-locus diversity.

This paper documents the distribution of *Ig* H chain alleles in populations of the Kerguelen archipelago where >99% of rabbits were homozygous for the *b4* allele of the *b* locus. An analysis of variance and the two-locus disequilibria are presented and compared to data previously obtained with populations that were polymorphic for both the *b* locus and *e* locus. We will address the hypothesis that the observations are due to the existence of compensatory diversity enhancing selection.

MATERIALS AND METHODS

Sample collection: Populations were sampled during Antarctic summers between 1984 and 1987. Collection sites refer to areas of 200 to 500 hectares, matching the average size of the small islands covered in this study. Rabbits were shot, except at locality PAF (*Port aux Français*), where live-traps were used. Blood clots and sera were stored and shipped

frozen. All rabbits analyzed were adults, as shown by eye lens weight and morphological criteria (BOUSSES *et al.* 1988). A detailed description of study sites and collection schemes can be found in BOUSSES (1991). The so-called "world-wide" sample consists of wild rabbits of Australia, Great Britain and continental Europe and was described in detail in VAN DER LOO (1993).

Alleles studied: The samples were tested for the following allotypic markers (for review see MAGE 1986): *b locus:* allotypes *b4, b5, b6, b9,* which are associated with multiple amino acid differences throughout the constant region of the L chain of the Kappa-1 class CK1. *a locus:* allotypes *a1, a2, a3* corresponding to multiple amino acid interchanges in the VHa variable region of the Ig H chain [Note: these changes affect mainly positions outside the antigen-binding regions (*CDR*)]. *d locus:* allotypes *d11* and *d12,* associated with a Met/Thr interchange at position 219 of the hinge region of the CG H chain region (*H* exon of the *CG* gene). *e locus:* allotypes *e14* and *e15,* associated with a Thr/Ala interchange at the position 309 of the CH2 domain of the CG region (*CH2* exon of the *CG* gene).

Determination of *Ig* allotypes: Antisera against a particular allotype were raised in rabbits according to KELUS and GELL (1960). Each serum sample was tested in the presence of a positive reference, as previously described (VAN DER LOO 1993). Due to allelic exclusion (RITCHIES *et al.* 1984), the two arrays of *a*, *d* and *e* allotypic markers present on the individual antibody reflect the allele combinations of the uniting gametes. The different H chain "haplotypes" can be determined by double gel immunodiffusion (KELUS and STEINBERG 1991) or by the use of antisera that recognize specific combinations of H chain markers (DUBISKI and GOOD 1972; HAMERS-CASTERMAN *et al.* 1979). We used antisera revealing, respectively, the *a1-d11* and the *a3-d11* combination.

Analysis of population variance: The parameters of population structure and chi-square values were estimated from variance components following COCKERHAM (1973) for subdivided populations. These components define the correlations or "degree of relationship" between pairs of genes (1) within individuals: F, (2) within individuals within divisions: f, and (3) of different individuals within the same division: θ. F estimates the overall inbreeding coefficient, f the inbreeding within populations and θ the degree of differentiation between populations. They are analogous to WRIGHT'S (1951, 1978) F_{IT}, F_{IS} and F_{ST}, respectively. The relative effect of subdivisions (S) within divisions (D) is measured by the correlations between pairs of genes (1) between individuals within the same subdivision (θ_1) and (2) between subdivisions within the same division (θ_2). F^{DS}, f^{DS} and θ^{DS} (f_3, f_2 and f_1 in COCKERHAM 1973) are F, f and θ for subdivision S, ignoring effects due to differences between divisions D.

Analysis of two-locus disequilibria: This was done following WEIR (1990). For two loci, with alleles A, a and B, b, the total deviation of genotype frequencies from expectations at Hardy-Weinberg equilibrium (HWE) based upon allele frequencies p_A and p_B is resolved into components that are due to (1) the one-locus disequilibria: D_A and D_B, (2) the gametic and nongametic digenic disequilibria: D_{AB} and $D_{A/B}$, respectively, (3) the trigenic disequilibria: D_{AAB} and D_{ABB}, and (4) the quadrigenic disequilibrium: D_{AABB}. Tri- and quadrigenic disequilibria will be referred to as *higher order* correlations: they measure associations among genes at two loci when all two-allele associations have been removed. Significance levels were estimated according to WEIR and COCKERHAM (1989). For the a locus (three alleles), three pairs of two-allele loci (a_i and non-a_i) were analyzed. $\Sigma_i D_{aiaiB}$ is the "total" trigenic association of allele B with homozygosity at locus a.

Correlation of heterozygosity levels between loci: The 10 two-locus genotypes can be classified as being *heterozygous* for either two, one or zero loci. Let P_2, P_1 and P_0 be the corresponding observed relative frequencies of these classes. The hypothesis of nonadditive or "compensatory" diversity enhancement predicts some negative correlations in heterozygote levels between the interacting loci (confirmed by simulations). One possible way of evaluating the plausibility of the working hypothesis consists of searching for higher order disequilibria that contribute to P_1, the fraction of single heterozygotes (or "heterotypes," in opposition to "homotypes," the double homo- and heterozygotes). This approach is somewhat similar to testing the hypothesis of overdominance-type selection by measuring heterozygote excess in the adult offspring generation and should present, at the very least, similar limitations (LEWONTIN and COCKERHAM 1959; *cf.* WEIR 1990, page 195).

According to WEIR (1990) the relative frequencies of genotype classes P_i ($i = 0, 1, 2$) for two loci with alleles A, a and B, b respectively, equals

$$P_i = f_i(p_A, p_B, D_A, D_B, D_{AB}, D_{A/B}) + T_i(D_{AAB}, D_{ABB}) + Q_i(D_{AABB}).$$

The fraction of double homozygotes P_0,

$$P_0 = P_{BB}^{AA} + P_{BB}^{aa} + P_{bb}^{AA} + P_{bb}^{aa},$$

can then be written as

$$P_0 = f_0 + T_0 + Q_0,$$

where

$$T_0 = T_{BB}^{AA} + T_{BB}^{aa} + T_{bb}^{AA} + T_{bb}^{aa},$$

$$Q_0 = Q_{BB}^{AA} + Q_{BB}^{aa} + Q_{bb}^{AA} + Q_{bb}^{aa}.$$

Following WEIR (1990, pp. 99–100)

$$T_{BB}^{AA} = 2p_B D_{AAB} + 2p_A D_{ABB},$$

$$Q_{BB}^{AA} = D_{AABB},$$

therefore

$$T_0 = 2p_B(D_{AAB} + D_{aaB}) + 2p_b(D_{AAb} + D_{aab}) + 2p_A(D_{ABB} + D_{Abb}) + 2p_a(D_{aBB} + D_{abb}),$$

$$Q_0 = D_{AABB} + D_{aaBB} + D_{AAbb} + D_{aabb}.$$

Because

$$D_{ABB} = D_{Abb} = -D_{aBB} = -D_{abb},$$

$$D_{AABB} = D_{AAbb} = D_{aaBB} = D_{aabb},$$

we obtain

$$T_0 = 4(p_A - p_a)D_{ABB} + 4(p_B - p_b)D_{AAB},$$

$$Q_0 = 4D_{AABB}.$$

It is easily shown that

$$T_1 = -2T_2 = -2T_0,$$

$$Q_1 = -2Q_2 = -2Q_0,$$

therefore

$$T_1 = -8(p_A - p_a)D_{ABB} - 8(p_B - p_b)D_{AAB},$$

$$Q_1 = -8D_{AABB}.$$

T_1 and Q_1 measure the relative contribution of the higher order disequilibria to the heterotype frequencies. For convenience, the quantities $P_1 - T_1$ and $P_1 - T_1 - Q_1$ will be referred to as "expected" values of the fraction of heterotypes

under tri-, and under tri- and -quadrigenic equilibrium (*TE* and *TQE*, respectively). In analogy with the definition of heterozygote deficiency *F* for a one locus system relative to HWE (*cf.* HEDRICK 1990), we define F^{TQ} as the deficiency in heterotypes relative to expectations at *TQE*. By writing $T_1 + Q_1$ as TQ_1,

$$F^{TQ} = 1 - P_1/(P_1 - TQ_1).$$

While *F* measures the correlation of genes (*i.e.*, of allelic states *A vs. a*) at one locus, F^{TQ} measures the correlation of "genotypes" (*i.e.*, of genotypic states *homo vs. heterozygosity*) between two loci. Negative values of F^{TQ} will be referred to as heterotype "excess" (relative to *TQE*). A chi-square statistic estimating the significance of the *effects* of higher order disequilibria on the relative distribution of genotype classes is given by

$$\chi^2 = N\{TQ_1^2/(P_1 - TQ_1) + TQ_2^2/(P_2 - TQ_2) + TQ_0^2/(P_0 - TQ_0)\},$$

d.f. = 1; *N* being the sample size.

In the case of the *a* locus (three alleles; a_i, $i = 1-3$) and *e* locus (two alleles; *E*, *e*), a more detailed analysis of the impact of disequilibria is obtained by considering six genotype classes: three *e* locus classes *EE*, *Ee* and *ee*, each class being subdivided into *a* locus homozygotes and heterozygotes. Their frequencies P_{AAEE}, P_{AAEe}, P_{AAee}, P_{AaEE}, P_{AaEe} and P_{Aaee} relate to frequencies of the two-allele systems (a_i / $nona_i$) as

$$P_{AA..} = \sum_i P(a_i a_i ..),$$

$$P_{Aa..} = \tfrac{1}{2} \sum_i P(a_i nona_i ..).$$

Effect of subdivision: For a sample consisting of *n* subdivisions, the contributions of genotypic disequilibria TQ_{1j} were calculated independently for each subsample *j*. The total effect of genotypic disequilibria *within* localities TQ_{1s} was determined as the weighted mean of TQ_{1j} [*i.e.*, $\Sigma_j w_j TQ_{1j}$, where $w_j = n_j/N$, n_j the number of rabbits sampled in population *j*; $N = \Sigma_j n_j$]. Its overall contribution to single heterozygote frequencies was estimated as

$$F_S^{TQ} = 1 - P_1/(P_1 - TQ_{1s}).$$

Effects of genotypic disequilibria *between* localities on heterotype frequencies were estimated by calculating these disequilibria for the total sample after removal of all effects due to tri- and quadrigenic disequilibria within localities.

RESULTS

All 1338 sera showed clear identity reactions in the *b4* anti-*b4* immunoassay. All were negative in the *b6* and *b9* specific assays. Five sera reacted with antisera raised against the *b5* allele. All five showed clear serological identity with the *b5*-reference serum. These *b5*/*b4* sera were from animals of the main island *Grande Terre*, collected within a radius of <2 km in the southern part of site ARM (*Armor*). For the studied H chain loci all allotypes known to occur in domestic breeds were observed.

Analysis of variance: The relative gene frequencies at the different study sites are shown in Table 1. Table 2 gives the analysis of variance for the sample divided into islands while gene correlations for the samples from the main island "*Grande Terre*" (Figure 1) are displayed in Table 3. The 15 stations of *Grande Terre* were grouped into four areas (as indicated in Table 1 and Figure 1). Areas were tentatively defined as parts of the island among which natural gene flow should be limited (for details see BOUSSES 1991). Within localities, the genotype frequencies fulfilled HW expectations, as indicated by the absence of significant correlations of gene pairs within individuals (f^s). For a fraction of the sample, individuals could be classified by sex. As shown in Table 4, the separate analysis of males and females revealed that for the male sample, correlations *f* were always positive and greater than for the sample consisting of females, where *f* values tend to be negative. Correlations θ were greater among females than among males (Table 4).

Digenic disequilibria: As in all other immunogenetic studies on rabbits, the *d11* marker was always found in association with the *e15* marker (MAGE 1982; VAN DER LOO 1987). Due to this apparent lack of recombination, *d* and *e* markers can define a single locus "*d-e*" or "*g*" with three alleles: *d11e15* (=*d11*), *d12e14* (=*e14*) and *d12e15*. Obviously, the frequency of the third allele, p_{d12e15}, equals $1 - p_{d11} - p_{e14}$. As in previous studies (VAN DER LOO and ARTHUR 1987), very strong linkage disequilibria were also observed between the variable region and the constant region genes. Only seven types of haploid combinations (haplotypes) of *a-d-e* locus alleles were observed at Kerguelen. Most common was haplotype *a3d12e15* (61.6% at Kerguelen *vs.* 18.3% for the world-wide study). The frequencies of the other haplotypes were as follows: *a1e14* (14.2% *vs.* 12.8%), *a2e14* (9.9% *vs.* 6.3%), *a3d11* (9.8% *vs.* 18.5%), *a2d12e15* (2.8% *vs.* 6.8%), *a3e14* (1.0% *vs.* 0.9%) and *a1d12e15* (0.6% *vs.* 27.2%). The quite common haplotype *a1d11* (7% in the world-wide sample) was completely absent.

We notice that at the gamete level both the *e15* and *e14* alleles appeared in combinations with each of the three *a* locus alleles, while the *d11* allotype was found only in association with the *a3* allele. Given a recombination frequency of 0.001 (SD 0.0003) between *a* locus and *d*/*e* loci (KELUS and STEINBERG 1991), the distribution of haplotype frequencies at Kerguelen was found compatible with the hypothesis that only the five more frequent types were represented in the small founder population that was released 120 years ago (*note:* a detailed analysis of the digenic disequilibria will be presented elsewhere).

Genotypic disequilibria: The nongametic digenic disequilibria differed significantly from zero and consis-

TABLE 1

Relative frequencies expressed as percentages of alleles of the rabbit *Ig*G L chain and H chain loci on the Kerguelen Islands

			a locus			*d* locus	*e* locus
			Allele				
Locality	Area	Sample size	*a1*	*a2*	*a3*	*d11*	*e14*
			Grande Terre (Main isle)				
RAT	A	42	16.67	—	83.33	15.48	15.48
CHA	A	15	40.00	—	60.00	6.67	40.00
MOR	A	69	14.49	—	85.51	14.49	13.77
MOL	A	86	20.35	5.81	73.84	0.58	22.09
PAF	A	118	13.56	0.42	86.02	1.27	13.56
ROC	A	52	20.19	—	79.81	—	20.19
ELI	A	46	8.70	5.43	85.87	2.17	13.04
STU	A	83	26.51	4.22	69.28	3.61	29.52
RON	B	69	58.70	18.84	22.46	—	77.54
PJA	B	43	33.72	22.09	44.19	—	47.67
COU	C	101	—	33.66	66.34	0.50	24.75
TRa	C	22	—	27.27	72.73	15.91	25.00
ARM	D	67	23.88	14.18	61.94	—	26.12
BOS	D	48	35.42	13.54	51.04	12.50	37.50
OBS	D	56	—	20.54	79.46	49.11	16.07
			Smaller islands				
COC	Cochon	46	15.22	—	84.78	56.52	15.22
CAT	Chat	32	18.75	23.44	57.81	—	29.69
CIM	Cimetière	146	—	—	100.00	—	—
GUI	Guillon	75	—	18.67	81.33	26.67	22.67
INS	Inskip	75	1.33	43.33	55.33	32.67	47.33
VER	Verte	36	—	38.89	61.11	—	38.89
HOW	Howe	11	31.82	9.09	59.09	—	40.91
Total		1338	14.91	12.74	72.35	9.83	25.07
Average		61	17.2	13.6	69.1	10.8	28.1

For identification of localities see Figure 1. Localities of the main island can belong to area A, B, C or D.

tently had the same sign as the gametic associations (not shown). Positive values of the quadrigenic disequilibria D_{aagg} were therefore always larger than their composite measures Δ_{aagg} ($D_{aagg} = \Delta_{aagg} + D^*_{ag}D_{a/g}$; WEIR 1990). Table 5 presents the genotypic disequilibria together with their relative contribution to the fraction of single heterozygotes. Higher order associations are also shown for a sample collected in areas where populations are polymorphic at the *b* locus (the socalled world-wide sample; detailed genotype frequencies of this sample are listed in Table 11 in VAN DER LOO 1993). A concise overview of the total trigenic disequilibria is given in Table 6.

Trigenic effects did always correspond to *a*/*e*-heterotype excess and to *a*/*d*-heterotype deficiency. Quadrigenic disequilibria among the H chain loci were significantly positive at Kerguelen. They did reduce, but not annihilate, the increase of *a*/*e*-heterotypes implemented by the trigenic correlations (Table 5). Patterns of disequilibria observed in the world-wide sample were similar to those at Kerguelen, but less pronounced in their effects. In former sample *e*-locus heterozygosity was furthermore associated with homozygosity at the *b* locus (*cf.* VAN DER LOO 1993). Within the Kerguelen sample, where virtually all rabbits were homozygous at this L chain locus, disequilibria involving the *b* locus cannot exist. In a previous report (VAN DER LOO 1993), the trigenic disequilibrium D_{BBE} was found to be negative for world-wide sample ($D_{BBE} = -0.003$, $\chi^2 = 4$; where *B* is *nonb5*). For the pooled sample (world-wide and Kerguelen) this correlation, which indicates an association between *b* locus heterozygosity (*Bb*) and the *e15* allele (*E*), was highly significant ($D_{BbE} = +0.008 = -2D_{BBE}$, $\chi^2 = 16$). Correlations D_{BEE} and D_{BBEE} were not significant, ($\chi^2 < 2$, not shown; because 97.5% of genes were either *b4* or *b5*, the *b* locus was treated as a two-allele locus with alleles *b5* and *nonb5*).

Subdivided sample: In an attempt to distinguish effects due to disequilibria *within* subdivisions from those due to variation *among* subdivisions, correlations were determined for the total sample after removal of all higher order disequilibria *within* (each of the 22) subdivisions (see MATERIALS AND METHODS). This is shown in Table 5 (bottom) for the *a*/*e*-locus interactions analyzed as

TABLE 2

Correlations of Ig H chain alleles on the Kerguelen Islands

Correlations[a] between gene pairs	Chi square	Allele *a1*	*a2*	*a3*	*d11*	*e14*
Nonhierarchical analysis						
Within individuals I						
F^S		0.216	0.172	0.164	0.332	0.151
	χ^2	*58.0*	*35.8*	*32.6*	*138.7*	*27.4*
Within I within localities S						
f^S		0.034	−0.002	−0.010	0.068	−0.018
	χ^2	*0.9*	*0.0*	*0.0*	*1.8*	*0.2*
Between I within S						
θ^S		0.189	0.174	0.173	0.284	0.166
	χ^2/d.f.	*30.9*	*28.7*	*27.0*	*33.3*	*24.6*
Between I within islands E						
θ^E		0.100	0.162	0.111	0.272	0.103
	χ^2/d.f.	*42.2*	*40.8*	*43.4*	*49.7*	*38.6*
Hierarchical analysis						
Localities S within islands E						
θ_2^{ES}		0.084	0.152	0.098	0.260	0.090
θ_1^{ES}		0.220	0.231	0.210	0.369	0.201
f^{ES}		0.034	−0.002	−0.010	0.068	−0.018
θ^{ES}		0.149	0.093	0.124	0.148	0.121
F^{ES}		0.178	0.091	0.115	0.206	0.106
Distribution of variance (%)						
Within individuals I						
$1-F^E$		75.3	77.0	79.8	58.8	81.4
Between I within S						
$F^E-\theta_1^{ES}$		2.6	−0.2	−0.8	4.3	−1.4
Between S within E						
$\theta_1^{ES}-\theta_2^{ES}$		13.7	7.9	11.2	10.9	11.0
Between E						
θ_2^{ES}		8.4	15.2	9.8	26.0	9.0

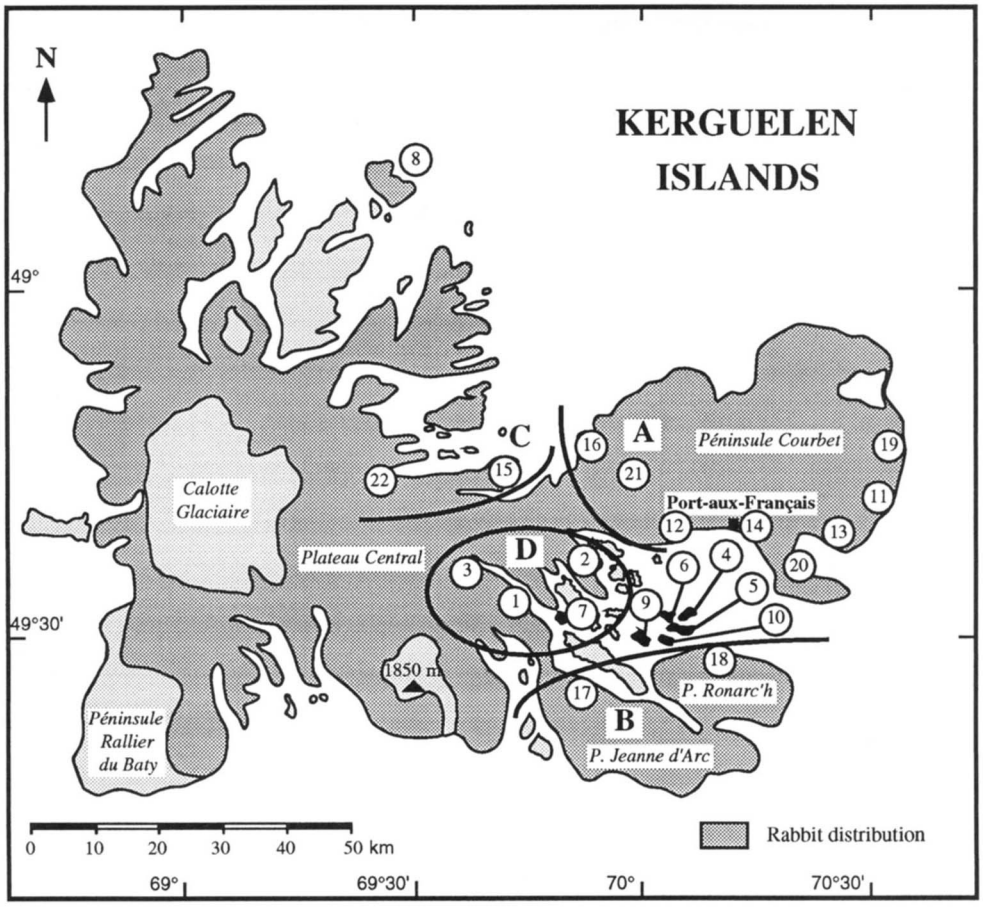
[a] Correlations between gene pairs for 2676 genes within 1338 rabbits within 22 localities within eight islands. Weighted by sample size after COCKERHAM (1973).

three two-locus/two-allele systems. It reveals that the amplitude of genotype disequilibria was only slightly diminished by this removal. Observed frequencies of genotype classes were found to satisfy expectations at *within-locality-TQE,* also when the analyses were carried out at the level of six a/e-locus genotype classes (Table 7). TQE was observed at most sites of the world-wide study. This is shown in Table 7 for the largest sample (Grassy Creek, 575 rabbits). At Grassy Creek, digenic correlations (a/de) were very strong (not shown) whereas θ values were negligible (Table 8). It seems that the strong effects of higher order correlations on heterotype frequencies observed for the Kerguelen sample are merely due to gene correlations *between* subdivisions (θ).

DISCUSSION

It is generally assumed that the primary cause underlying the unusual degree of allelic variation at *Ig* loci is preventing the emergence of immune-avoidance mechanisms in parasites (*cf.* HOWARD 1991). This paper addresses the question whether selective advantage of gene diversity is compensatory rather than additive among the different loci that contribute to the Ig protein complex. A more specific working hypothesis is based upon the observations that (1) in the aboriginal area of distribution, where diversity at *a* and *b* loci is extensive, all individuals were homozygous for the *e15* allele and (2) in areas where populations were polymorphic at the *e* locus, heterozygosity excess at the *b* locus was associated with *e15* homozygosity (see Introduction). This may suggest that *b* locus and *a* locus diversity evolved in the absence of *e* locus polymorphism, while the *e* locus polymorphism (that is to say, the *e14* allele) evolved within the context of reduced levels of gene diversity at both the *a* and the *b* locus. As a consequence, forces enhancing diversity at the *b* and/or at the *a* locus could be correlated in strength to the degree of *e15* homozygosity.

Evaluation of the nonadditive/compensatory hypothesis from population data: Different formulations of the above hypothesis predict some negative correlation in heterozygosity levels among the *Ig* loci. In this study we first



1. Armor (ARM)
2. Baie de l'Observatoire (OBS)
3. Fjord Bossière (BOS)
4. Ile du Chat (CAT)
5. Ile du Cimetière (CIM)
6. Ile aux Cochons (COC)
7. Ile Guillou (GUI)
8. Ile Howe (HOW)
9. Ile Inskip (INS)
10. Ile Verte (VER)
11. Pointe Charlotte (CHA)
12. Pointe Molloy (MOL)
13. Pointe du Morne (MOR)
14. Port-aux-Français (PAF)
15. Port-Couvreux (COU)
16. Port-Elisabeth (ELI)
17. Port-Jeanne-d'Arc (PJA)
18. Presqu'île Ronarc'h (RON)
19. Ratmanoff (RAT)
20. Roche Verte (ROC)
21. Val Studer (STU)
22. Val Travers (TRA)

FIGURE 1.—Map of the Kerguelen archipelago (subantarctic, 49°S, 70°E). Numbering of collection sites is in alphabetic order. Their grouping into areas A, B, C, and D are indicated.

looked for correlations at the *population* level and then we attempted to trace back these correlations to the effects at the *individual* level. The *first* approach consisted of (1a) measuring the degree of gene diversity at Kerguelen (devoid of *b* locus diversity) and comparing this to that reported previously for areas where diversity was substantial at both the *b* locus and *e* locus, and (1b) estimating the two-locus disequilibria in the two undivided samples while verifying the specific contribution of genotype-dependent disequilibria to single-heterozygosity levels. The *second* approach consists of (2a) searching for evidence of heterozygous excess at H chain loci in the subdivided Kerguelen sample and (2b) testing how far the relevant genotypic disequilibria in the total sample are due to effects within the subdivisions. Specific attention was paid to the question whether *a* locus heterozygosity is correlated to *e15* homozygosity.

Constitutive *Ig* allele diversity at Kerguelen: Compared to frequencies reported from other areas, the Kerguelen populations differ by (1) absence of *b* locus diversity; (2) a pronounced frequency hierarchy at the *a* locus ($p_{a3} \gg p_{a1} > p_{a2}$; $p_{a3} = 0.69$); (3) relatively high frequencies of the *e14* allele, particularly if the strong negative gametic association between *a3* and *e14* alleles is taken into account. The data must be viewed within the context of the known history of rabbit colonization in the Kerguelen archipelago. The current feral populations are descendants of some 20–50 rabbits that were released on the archipelago during the year 1874 (KIDDER 1876). These rabbits were themselves collected at Table Bay Island (EATON 1879), off the coast of South Africa and were descendants of domestic rabbits of Dutch origin. Such a succession of population bottle-necks could explain a more reduced number of H chain founder haplotypes and the loss of the *b* locus polymorphism. The presence of five rabbits expressing the *b5* allotype at Armor is puzzling. It could be related to the presence, four decades ago, of a center of human activity in this area (*cf.* BOUSSES 1991) and may reflect the (limited) effect of an undocumented introduction.

TABLE 3

Correlations of genes on Kerguelen Main Island

Correlations[a] of genes	Chi-square	Allele *a1*	*a2*	*a3*	*d11*	*e14*
Nonhierarchical analysis						
Within individuals I						
F^S		0.184	0.151	0.159	0.311	0.157
	χ^2	*15.2*	*35.7*	*20.0*	*75.1*	*17.7*
Within I within localities S						
f^S		0.032	0.019	0.006	0.085	0.005
	χ^2	*0.7*	*0.1*	*0.0*	*1.8*	*0.0*
Between I within S						
θ^S		0.157	0.134	0.153	0.248	0.153
	χ^2/d.f.	*18.7*	*30.2*	*23.7*	*52.3*	*25.6*
Between I within areas A						
θ^A		0.1501	0.181	0.171	0.099	0.168
	χ^2/d.f.	*73.4*	*125.5*	*81.1*	*160.4*	*104.1*
Hierarchical analysis						
$\theta_2{}^{AS}$		0.139	0.180	0.164	0.057	0.161
$\theta_1{}^{AS}$		0.195	0.185	0.198	0.262	0.197
f^{AS}		0.032	0.019	0.006	0.085	0.005
θ^{AS}		0.065	0.005	0.041	0.217	0.043
F^{AS}		0.095	0.025	0.047	0.283	0.048
Distribution of variance (%)						
$1-F^A$		77.9	80.0	79.6	67.6	79.9
$F^A-\theta_1{}^{AS}$		2.6	1.6	0.5	6.3	0.4
$\theta_1{}^{AS}-\theta_2{}^{AP}$		5.6	0.4	3.5	20.5	3.6
$\theta_2{}^{AS}$		13.9	18.0	16.4	5.7	16.1

[a] Correlations between gene pairs for 1834 genes within 917 individuals within 15 localities within four areas within the southwestern part of the main island. Weighted by sample size after COCKERHAM (1973).

Distribution patterns of H chain diversity in absence of L chain diversity: Differentiation among localities was important and contributed 20–40% to the total heterozygosity (Tables 2 and 3). This pronounced differentiation was not only observed for islands: for the *a*- and *e*-locus alleles, genetic divergence among localities of the main island (θ^S in Table 3) was even greater than among islands (θ^E in Table 2). The strong divergence at the H chain loci contrasts with earlier reports. In Table 8, the gene correlations on Kerguelen are compared to those previously reported for wild populations from other areas. In the latter samples, parameters of genetic differentiation among divisions and subdivisions were rather marginal as far as the H chain loci were concerned (for details see VAN DER LOO 1993).

It should be noted that genetic differentiation, as mea-

TABLE 4

Correlations of genes for male and female rabbits

Correlations of genes	Chi-square	Allele *a1*	*a2*	*a3*	*d11*	*e14*
Nonhierarchical analysis						
Between rabbits within localities θ^S						
Male (n = 328)		0.162	0.122	0.128	0.152	0.125
	χ^2/d.f.	*12.4*	*7.8*	*7.4*	*7.6*	*7.3*
Female (n = 436)		0.191	0.206	0.147	0.215	0.179
	χ^2/d.f.	*12.8*	*15.5*	*9.6*	*12.9*	*12.4*
Within rabbits within localities f^S						
Male (n = 328)		0.224	0.032	0.114	0.127	0.110
	χ^2	*10.0*	*0.3*	*3.8*	*3.8*	*3.5*
Female (n = 436)		−0.02	0.045	−0.006	−0.073	−0.072
	χ^2	*0.07*	*0.6*	*0.0*	*1.1*	*1.0*

TABLE 5

Higher order disequilibria among rabbit Ig loci and their contribution to heterotype frequencies

Allele at locus		Coefficient of disequilibrium			Effects			Heterotype deficiency
a	*g*	D_{aag}	D_{agg}	D_{aagg}	T_{1g}	T_{1a}	T_1+Q_1[a]	F^{TQ}[b]
			World-wide (1539 rabbits)					
a1	*d11*	−0.0024[c]	−0.0084****	+0.0056****	−0.009	−0.004	−0.058	**+0.125*****
a2	*d11*	−0.0020	−0.0010	+0.0001	−0.008	−0.006	−0.015	+0.035
a3	*d11*	−0.0005	+0.0096****	+0.0064	−0.002	+0.016	−0.037	**+0.097****
a1	*e14*	+0.0041*	−0.0006	+0.0036**	+0.019	0.000	−0.010	+0.022
a2	*e14*	+0.0056***	+0.0006	0.0004	+0.027	+0.004	+0.027	**−0.091****
a3	*e14*	+0.0007	−0.0000	−0.0003	+0.003	0.000	+0.006	−0.012
b4	*e14*	+0.0030*	−0.0016	−0.0010	+0.014	+0.004	+0.027	**−0.063***
b5	*e14*	+0.0033*	+0.0020	+0.0003	+0.016	+0.007	+0.019	−0.048
b9	*e14*	−0.0002	−0.0004	−0.0005	−0.001	−0.003	+0.000	−0.001
			Kerguelen (1338 rabbits)					
a1	*d11*	−0.0014*	−0.0035***	−0.0002	−0.009	−0.020	−0.027	**+0.087***
a2	*d11*	−0.0021**	−0.0039****	+0.0002	−0.014	−0.023	−0.038	**+0.137*****
a3	*d11*	−0.0017*	+0.0074****	+0.0013**	−0.011	−0.027	−0.048	**+0.116******
a1	*e14*	+0.0165****	+0.0113****	+0.0105****	+0.066	+0.064	+0.048	**−0.364******
a2	*e14*	+0.0085****	+0.0008	+0.0063****	+0.034	+0.005	−0.013	+0.048
a3	*e14*	+0.0119****	−0.0121****	+0.0019****	+0.048	+0.043	+0.053	**−2.297******
			Disequilibria between localities at Kerguelen (22 sites)					
a1	*e14*	+0.0140	+0.0079	+0.0083	+0.056	+0.044	+0.031	**−0.240**
a2	*e14*	+0.0094	+0.0035	+0.0071	+0.038	+0.021	+0.003	−0.009
a3	*e14*	+0.0106	−0.0114	+0.0065	+0.043	+0.041	+0.037	**−2.040**

Effects are shown only for two-locus systems where higher order disequilibria were significant. F^{TQ} measures the correlation of heterozygosity levels between loci. The world-wide sample represents 16 populations from Australia, Great Britain and continental Europe. For the Kerguelen sample, *a*/*e*-locus disequilibria between localities were estimated as the disequilibria in the total sample after removing all effects due to tri- and quadrigenic correlations within localities.

[a] $T_{1g} = -8(2p_g-1)*D_{aag}$; $T_1 = T_{1g}+T_{1a}$; $Q_1 = -8D_{aagg}$.

[b] $F^{TQ} = 1 - P_1/(P_1 - T_1 - Q_1)$, where P_1 is the fraction of single heterozygotes (heterotypes).

[c] Probability $D = 0$: $*P < 5\%$; $**P < 1\%$; $***P < 0.1\%$; $****P < 0.01\%$.

sured at H chain loci, was much more pronounced among the 15 localities from the southeastern part of the main island than among 16 wild populations collected world-wide. The differences between θ_1 for the world-wide sample (0.025–0.09) and θ_1 for Kerguelen (0.20–0.37) are very large indeed. At the *b* locus, the opposite situation prevailed. On Kerguelen virtually all rabbits were homozygous for this locus ($H_p = 0$ and $\theta_1 = 0$) whereas levels of heterozygosity were consistently high for populations of the world-wide sample and the interlocality variance comparatively great ($H_p = 0.41 \pm 0.14$ and $\theta_1 = 0.13$; see VAN DER LOO 1993). In the light of existing indications of negative interferences between gene diversity at H and L chain (see Introduction), one might be justified in asking whether the strong genetic variance at the H chain loci among Kerguelen sites might be connected to the lack of *b* locus diversity.

Evidence for heterozygous excess: The observation of HWE on Kerguelen is surprising. The behavioral patterns of the rabbit (stable territorial groups with separate linear dominance hierarchies among males and females) are expected to cause some level of inbreeding within collection sites (DALY 1979; RICHARDSON 1981; BELL 1983). In previous studies, inbreeding coefficients *f* measured at the *Ig* H chain loci varied between 0.04 and 0.14 (Table 8). These values appeared to be affected by the way in which populations were sampled. In samples collected by shooting rabbits over short periods of time, as was the case here, *f* values at the *a* locus were always higher than 0.10 (HERD and EDMONDS 1977; VAN DER LOO 1993). In this study, collection sites corresponded to areas of 200–500 hectares, which is much larger than the average size of the 11 subareas (<5 hectares) of the Grassy Creek site referred to in Table 8. There are therefore two reasons for expecting a higher degree of genetic variation (or inbreeding) within "localities" at Kerguelen: (1) areas covered by localities were comparatively larger and (2) genetic differentiations between sites much greater than in previous studies. Instead, taken at face value, the absence of gene correlations *f* indicate genetic homogeneity within the Kerguelen sites.

TABLE 6

Total trigenic disequilibria

Correlation of *d-e* (*g*) haplotypes with homozygosity at either *a* or *b* locus		
World-wide		
d11e15	$\Sigma_j D_{ajajg1} = -0.0050$	$\Sigma_j D_{bjbjg1} = -0.0048$
d12e15	$\Sigma_j D_{ajajg2} = -0.0054$	$\Sigma_j D_{bjbjg2} = -0.0013$
d12e14	$\Sigma_j D_{ajajg4} = +0.0104$	$\Sigma_j D_{bjbjg4} = +0.0060$
Kerguelen		
d11e15	$\Sigma_j D_{ajajg1} = -0.0052$	
d12e15	$\Sigma_j D_{ajajg2} = -0.0317$	
d12e14	$\Sigma_j D_{ajajg4} = +0.0369$	
Correlation of a locus allotypes with homozygosity at *d-e* (*g*) locus or *b* locus		
World-wide		
a_1	$\Sigma_j D_{gjgja1} = -0.0131$	$\Sigma_j D_{bjbja1} = -0.0051$
a_2	$\Sigma_j D_{gjgja2} = -0.0045$	$\Sigma_j D_{bjbja2} = +0.0067$
a_3	$\Sigma_j D_{gjgja3} = +0.0175$	$\Sigma_j D_{bjbja3} = -0.0016$
Kerguelen		
a_1	$\Sigma_j D_{gjgja1} = +0.0087$	
a_2	$\Sigma_j D_{gjgja2} = -0.0027$	
a_3	$\Sigma_j D_{gjgij3} = -0.0060$	

d-e-locus haplotypes were treated as alleles of one locus (*g*) with alleles g_1 (*d11e15*), g_2 (*d12e15*) and g_4 (*d12e14*). The total trigenic association $\Sigma_j D_{ajajgi}$ is the sum of the trigenic disequilibria between the g_i allele and all classes of *a*-locus homozygotes and measures the association of a particular allele at one locus with homozygosity at a second locus. Trigenic correlations of the *e15*-sharing haplotypes g_1 and g_2 with either *a*- or *b*-locus homozygosity were found to be consistent in sign.

TABLE 7

Contribution of higher order disequilibria within localities to *a/e* genotypic frequency classes

Genotype classes	Observed numbers	Expected numbers	F_S^{TQ}	$\Sigma_j \frac{(E_j - O_j)^2}{E_j}$
Grassy Creek, undivided (n = 575)				
NP_0	189	185.96		
NP_1	294	300.08	0.02	0.3
NP_2	92	88.96		
Kerguelen undivided (n = 1338)				
NP_0	832	859.4		
NP_1	100	45.2	−1.2	68.9
NP_2	406	433.4		
Kerguelen, 22 subdivisions				
NP_0	832	836.73		
NP_1	100	90.65	−0.10	1.0
NP_2	406	410.63		
NP_{AAee}	97	95.48		
NP_{AAEE}	735	741.25		
NP_{AAeE}	23	18.27	−0.26	
NP_{aAee}	23	24.46	0.06	2.2
NP_{aAEE}	54	47.91	−0.13	
NP_{aAeE}	406	410.63		

P_0, P_1 and P_2 are the frequency classes of double homozygotes, single heterozygotes (or heterotypes) and double heterozygotes, respectively; P_{AA}.. are the frequencies of *a*-locus homozygotes, and P_{aA} of heterozygotes, within a particular *e*-locus genotype class; *E* stands for *e15*, *e* for *e14*. For subdivided populations, "expected numbers" refer to tri- and quadrigenic equilibrium within each of the subdivisions; F_S^{TQ} measures the correlation of heterozygosity between loci after removal of all effects due to disequilibria between subdivisions.

A possible explanation is that at Kerguelen the effect of local inbreeding ($f > 0$) was counterbalanced by effects of processes that increase heterozygote frequencies ($f < 0$), such as overdominant-type selection. The differences in θ and f between sexes, reported in Table 4, are supportive of this hypothesis. In males, correlations f between gene pairs were *significantly* positive for some alleles and always stronger than in females where they tend to be negative. Because correlations θ were more pronounced among females (which indicates that differentiation between localities was greater for females than for males), the observed sex differences in population parameters can be explained by male-specific dispersion (*cf.* WEBB *et al.* 1995). This implies that males tend to represent larger areas than females. Such sex differences in allele correlations were not observed for Australian populations that were collected in areas where the genetic differentiation at the studied loci was much more limited (similar sample sizes, W. VAN DER LOO and B. J. RICHARDSON, unpublished results). The higher level of f recorded for males at Kerguelen therefore constitutes strong evidence for Wahlund effect as a source of homozygosity excess. It appears that we might be witnessing a situation that is reminiscent of the study by HERD and EDMONDS (1977), where according to VAN DER LOO (1993) a 10% excess heterozygosity at the *b* locus went undetected because of the genetic differentiation within collection sites (*cf.* Table 8).

Negative interlocality correlations in diversity levels between *Ig* H chain and L chain loci: In the aboriginal Mediterranean range of the species, *b* locus diversity was extensive (>10 alleles, VAN DER LOO *et al.* 1991) while all populations were fixed for the ubiquitously predominant *e15* allele ($H_T(b) > 87\%$, $H_T(e) = 0\%$). In the world-wide sample with only two or three alleles at the *b* locus, the *e* locus was polymorphic ($H_T(b) = 48\%$; $H_T(e) = 32\%$) and heterozygote excess at the *b* locus was found associated with *e15* homozygosity (*cf.* Table 6,9 in VAN DER LOO 1993). On Kerguelen, populations were fixed for the preponderant *b* locus allele

TABLE 8

Gene correlations θ and f at Ig loci in natural populations of rabbit: comparisons of different sample studies

		Allotype							
	Correlation	*b4*	*b5*	*b9*	*a1*	*a2*	*a3*	*d11*	*e14*
Victoria, Australia[a]									
Rabbits (n = 5384)	f^S	0.046	0.061	0.038	0.144	0.135	0.137	ND	ND
Divisions D (n = 6)	$\theta_2{}^{DS}$	0.015	0.013	0.012	−0.001	0.008	0.002	ND	ND
Subdivisions S (n = 25)	$\theta_1{}^{DS}$	0.041	0.039	0.034	0.059	0.020	0.053	ND	ND
Australia, Great Britain, Continental Europe[b]									
Rabbits (n = 1539)	f^S	−0.048	−0.053	0.001	0.042	0.112	0.082	0.095	0.040
Divisions D (n = 3)	$\theta_2{}^{DS}$	−0.067	−0.019	−0.020	0.051	0.008	0.004	−0.031	−0.018
Subdivisions S (n = 16)	$\theta_1{}^{DS}$	0.152	0.078	0.224	0.089	0.076	0.066	0.047	0.025
Grassy Creek[c]									
Rabbits (n = 575)	f^D	−0.073	−0.104	−0.045	0.034	0.069	0.036	0.091	0.021
Divisions D (n = 11)	θ	−0.006	−0.004	0.029	0.009	0.005	0.004	0.009	0.042
Kerguelen Archipelago									
Rabbits (n = 1338)	f^S	—	—	—	0.034	−0.002	−0.010	0.068	−0.018
Divisions D (n = 8)	$\theta_2{}^{DS}$	—	—	—	0.084	0.152	0.098	0.260	0.090
Subdivisions S (n = 22)	$\theta_1{}^{DS}$	—	—	—	0.220	0.231	0.210	0.369	0.200
Kerguelen Main isle									
Rabbits (n = 917)	f^S	—	—	—	0.032	0.019	0.006	0.085	0.005
Division D (n = 4)	$\theta_2{}^{DS}$	—	—	—	0.139	0.180	0.164	0.057	0.161
Subdivisions S (n = 15)	$\theta_1{}^{DS}$	—	—	—	0.195	0.185	0.198	0.262	0.197

θ_1 measures the differentiation among subdivisions within divisions, θ_2 that among divisions. f is the inbreedings coefficient estimated by the correlation of gene pairs within individuals within subdivisions. $f < 0$ indicates that heterotic effects are overriding Wahlund effect. The lower values of f for H chain alleles at Kerguelen can be explained by less variation within subdivisions (less inbreeding) or by stronger heterotic effects. ND, not determined.

[a] Calculated from data reported in HERD and EDMONDS (1979).

[b] From VAN DER LOO (1993).

[c] Grassy Creek is one of the 16 localities under[b].

(*b4*), whereas *e*-locus diversity was greater than in the world-wide sample ($H_T(b) = 0\%$; $H_T(e) = 40\%$). The data of Kerguelen when compared to those obtained for Australia, Great Britain and continental Europe reveal a *negative* interlocality correlation in heterozygosity among these *Ig* loci. This component of variation (which obviously cannot be measured in the Kerguelen sample separately) is accounted for by the negative correlation of *b* locus homozygosity with the *e15* allotype (D_{BBE}) in the pooled data set. Its value is highly significant. Because *e15* frequencies are substantially larger than *e14* frequencies, this corresponds to an excess in *b*/*e* heterotypes relative to TE.

In conclusion, the high degree of genetic differentiation in the Kerguelen sample, the comparatively high level of *e*-locus diversity, and the excess heterozygosity, could reflect effects of forces tending to enhance gene diversity at the H chain loci (among populations and within individuals). Such effects were not observed at the H chain loci in populations where diversity was apparently enhanced at the L chain *b* locus. The situation observed therefore meets expectations assuming that genetic variability at Ig H and L chain constant regions was sustained by compensatory diversity enhancing selection.

Genotypic disequilibria between loci of the constant and variable region of the H chain: While diversity-enhancement selection could clearly explain excess heterozygosity *within* populations, it is not obvious how it could relate to an increase in genetic differentiation *among* populations. A comparison of the trigenic disequilibria reveals a systematic correlation of the *e14* allotype with homozygosity at the *b*- and at the *a* locus (Tables 5–7). The *e*/*a*-locus disequilibria were particularly pronounced in the Kerguelen sample. In view of previous indications of correlations between the diversity levels at different *Ig* loci, strong genotypic disequilibria between the loci coding for the variable and the constant region, respectively, might have some meaning. We have analyzed them more in detail.

Undivided sample: The quadrigenic disequilibria are a predictable outcome of combined effects due to strong linkage disequilibria and genetic differentiation within the sample (Tables 2 and 3). This is easily understood if one considers the H chain *a-d-e* haplotypes as alleles at a single locus: the predicted homozygosity excess due to Wahlund effect will indeed be measured as heterotype deficiency in two-locus analyses. No such simple explanation exists for the trigenic disequilibria. For both sample studies, the *e14* allele was always involved in trigenic disequilibria that support heterotype excess (with both *b* and *a* loci) whereas the opposite was found for the *d11* allele (Table 5). When *d-e* haplotypes were treated like alleles, it appeared that *CG* haplotypes shar-

ing an *e*-locus marker (*d11e15* and *d12e15*) were always involved in trigenic disequilibria bearing the same sign (Table 6). This was clearly not the case for *CG* haplotypes that share a *d*-locus marker (*d12e15* and *d12e14*). It suggests that the underlying forces, if any, are in the first place concerned with the *e* locus markers.

At Kerguelen, the trigenic disequilibria were reinforced in their effects as a consequence of the exceptionally high frequency of the *a3* allele (Table 5). The impact of trigenic disequilibria on heterotype frequencies depends indeed heavily upon the differences in allele frequencies [*i.e.*, $T_{1A} = -8(p_A - p_a)D_{ABB}$]. Overdominance tends to reduce these differences and for this reason could weaken the trigenic effects in two-locus systems. It is therefore interesting that previous studies revealed consistent hierarchies in allele frequencies at the studied loci, with leading alleles (*i.e., b4, nona2, d12, e15*) at frequencies well above 60%. At least for the *a* and *b* loci there is good evidence that these frequency hierarchies are maintained by selection. Indeed, within the heterozygous individual, the more frequent alleles are also more expressed (*allelic imbalance;* LUMMUS *et al.* 1967; MAGE 1987; discussed in VAN DER LOO 1987, 1993). The observed positive digenic associations between the predominant alleles are interesting because, in absence of diversity enhancing forces, this situation should favor the loss of polymorphism. The hypothesis that the pronounced trigenic effects at Kerguelen could have to do with compensatory aspects of such forces would seem more plausible if higher order disequilibria in the total sample were found to be the accumulation of convergent effects *within* localities and *between* localities.

Genotypic disequilibria within subdivisions: The analysis of the subdivided sample revealed that the higher order effects recorded in the undivided samples (Tables 5 and 7) were almost entirely due to genotype correlations *between* localities or populations. We note nevertheless that, at Kerguelen, in agreement with the restricted working hypothesis, the largest overall deviations from within-locality TQE relate to an increase of *a* locus heterozygotes among homozygous *e15* rabbits (13%) and of *e* locus heterozygotes among *a* locus homozygotes (26%). The observed 10% excess of *a/e*-heterotypes within populations, although not significant, does certainly not underscore reasonable expectations of (nonadditive) diversity enhancing forces. However, because of the low expected heterotype frequencies in this area (4–8% for *a/e*-locus heterotypes, Table 7), larger samples would be required for a confirmation of a heterotype excess within localities of Kerguelen.

These low (expected) heterotype frequencies, which may seem contradicting the working hypothesis, are the direct consequence of the strong positive association between the leading alleles *a3* and *e15* (*i.e.*, the frequency of the *a3e14* haplotype is almost 20 times smaller than the product of the frequencies of the constituent allotypes). Linkage disequilibria between the closely linked *a* and *e* loci are a predictable outcome of founder effects in view of the recent history of most rabbit populations. Whereas higher order disequilibria were generally not significant, strong digenic disequilibria between these loci were found for all populations studied until now (DUBISKI and GOOD 1972; MAGE *et al.* 1982; VAN DER LOO 1987). More in particular, lineages carrying the *a3e14* haplotype were found neither in domestic breeds (MAGE *et al.* 1982; KELUS and STEINBERG 1991) nor in wild populations of Great Britain and Australia (VAN DER LOO and ARTHUR 1987, unpublished results). There are therefore good reasons to assume that the *a3e14* type did not occur in the founders of the Kerguelen population. However, ~4% of *e14* genes in this area were found on *a3* chromosomes. In one locality (COU) the *a3e14* frequency reached 6% (not shown). A closer analysis revealed that the two haplotypes *a3e14* and *a1d12e15*, which apparently derive from recombination events *in situ* (Kerguelen), did contribute to most of the observed 10% heterotype excess (*cf.* Table 7).

In summary, the pronounced effects of the higher order disequilibria on heterotype frequencies recorded at Kerguelen were shown to be largely, if not entirely, the outcome of the particular pattern of gene distribution. In essence, there is pronounced interlocality variation within a context of hierarchical allele frequencies, strong linkage disequilibria and most likely, overdominance. We remain uncertain about the contribution of higher order correlations within populations. Larger and more appropriate samples and adequate statistical tests will be needed to verify that the genotype correlations here observed are determined by genotypic interactions within individuals. We note nevertheless that (1) the gene distribution is such that the resulting trigenic effects in the total sample supports a highly significant fraction of *a* locus heterozygotes among *e* locus homozygotes and of *e* locus heterozygotes among *a* locus homozygotes; and (2) no such effects were observed for populations where *b* locus diversity was substantial.

Concluding remarks: Rabbits have been successfully introduced over the last 1000 years into most of the temperate zones and their presence was documented for more than 500 islands (FLUX and FULLAGAR 1986). The present study indicates that the analysis of populations where *Ig* allele diversity is restricted for historical reasons, can contribute to our understanding of the forces that sustain this diversity. At present the data reported concur with existing indications of negative correlations of gene diversity among *Ig* loci and seem to support the hypothesis that avoidance of total allotypic homogeneity at the immunoglobulin protein complex is the principal concern of the putative diversity enhancing mechanisms.

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