Proc. Natl. Acad. Sci. USA
Vol. 84, pp. 3075–3079, May 1987
Population Biology

Nonrandom allele associations between unlinked protein loci: Are the polymorphisms of the immunoglobulin constant regions adaptive?

(immunoglobulin allotypes/linkage disequilibrium/evolution/myxomatosis)

WESSEL VAN DER LOO*, CHRISTIAN P. ARTHUR†, BARRY J. RICHARDSON‡, MARIJKE WALLAGE-DREES§, AND RAYMOND HAMERS*

*Instituut voor Moleculaire Biologie, Vrije Universiteit Brussel, 1640 St-Genesius-Rode, Belgium; †Office National de la Chasse, Centre St-Benoit, 78610 Auffargis, France; ‡Department of Population Biology, Australian National University, Canberra, A.C.T. 2601, Australia; and §Departement voor Populatie Biologie, Vrije Universiteit Leiden, 2300 RA Leiden, The Netherlands

Communicated by Jean Brachet, November 3, 1986 (received for review July 1, 1986)

ABSTRACT **Consistent linkage disequilibrium was observed between independently segregating protein loci. In natural populations of the European rabbit *Oryctolagus cuniculus*, highly significant, nonrandom associations between alleles of the constant regions of the immunoglobulin light and heavy chains were found, both within localities and between localities. We suggest that the population genetic data presented here are relevant to the adaptive significance of the genetic polymorphisms of the antibody constant regions.**

An antibody molecule is composed of two types of polypeptide chains, the immunoglobulin (Ig) heavy (H) and light (L) chains, each type consisting of a variable (V) region and a constant (C) region. The V regions are involved in the recognition and targeting of the (foreign) antigen, while the Ig C regions mediate the antibody functions. In most species studied, including man, genetic polymorphisms have been observed at loci controlling the Ig C regions. Since their discovery (1), the structural and genetic bases of these so-called "Ig allotypes" have been extensively studied, often revealing an unusual degree of complexity (reviewed in ref. 2).

It is generally agreed that the special genomic mechanisms underlying the variability of the antibody V regions are the outcome of an adaptive process. The biological significance of the genetic diversity of the C regions is, however, not understood. Extensive genetic diversity could, in multiloci systems, be generated by purely random processes (3, 4), but the fact that microorganisms often manipulate the host's immune response by interacting with the C regions of the antibody has suggested that genetic diversity at this level might be part of an immune defense strategy and could affect survival (5).

In the European rabbit (*Oryctolagus cuniculus* L.), serologically defined polymorphisms have been described for both the H chain and L chain (1, 2). As in other species, H- and L-chain polymorphisms segregate as unlinked codominant Mendelian alleles. The different *b*-locus allotypes (b4, b5, b6, and b9) are associated with multiple amino acid differences throughout the C region of the κ L chain. Although alleles can differ by up to 33% in amino acid sequence (6, 7), studies at the genome level have confirmed them as true alleles at a single gene locus (8, 9). A most interesting finding was that the nucleotide sequences of *b*-locus alleles differ almost exclusively by base-pair substitutions causing amino acid replacements (7–9).

The two allelic forms *e14* and *e15* of the *e* locus, which controls the C region of the IgG H chain differ by only one amino acid change (10). Whereas *b*-locus allotypes seem to evolve very rapidly, phylogenetic data indicate that the serological markers of the *e*-locus polymorphisms have been conserved in evolution (11–15).

These observations might suggest that in rabbit, Ig allotypes are the outcome of adaptive evolution. However, the actual adaptive importance of genetic variation in populations can only be assessed by studying the extent to which the relative survival and reproductive success of individuals are affected by their genotype. Since differences in fitness among genotypes should be important in determining their relative frequencies, indirect evidence for selection might be obtained from the distributions of the relevant alleles in populations, provided that the selection force is relatively strong and that the sample sizes are sufficiently large (16, 17). We have used this approach to investigate the adaptive significance of the Ig C-region allotypes in wild populations of the European rabbit, a species that is exposed to an exceptionally virulent infectious disease, myxomatosis (18, 19).

MATERIALS AND METHODS

Localities. Field collections were made in Southwest Australia (populations 1–3), Eastern Australia (populations 4–9), Great Britain (populations 10–13; including Scotland, Northumberland, and Sussex), and continental Europe populations (14–16; Holland, Limburg, and Ile de France). In all areas myxomatosis was endemic.

Sample Collection. Most rabbits were live-trapped, marked, and released after 1–5 ml of blood was taken by cardiac puncture or from the marginal ear vein. In one locality (population 9) about 80% of the individuals were sampled, and their association to a particular warren system was recorded. At other places (populations 13, 14, and 15) the animals were shot, and blood was collected before rigor mortis. Serum was separated and stored at −20°C.

Typing. Typing of the IgG allotypes was done by immunodiffusion (1, 2, 20). Each sample was compared against positive standard sera. The antisera were produced as described (20) and were specific for the allotypes a1, a2, and a3

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: Ig, immunoglobulin; H and L chain, Ig heavy and light chain; V and C region, Ig variable and constant region; LD, linkage disequilibrium.

(*a* locus of the H-chain V region); b4, b5, b6, and b9 (*b* locus); e14 and e15 (*e* locus).

Data Bank. Allotypes were determined for 2000 rabbits on a first-come, first-served base. All data were included in this study except those from juvenile rabbits weighing <600 g, since these could contain traces of maternal Ig.

RESULTS

For the C region allotypes, only alleles known in domestic breeds were observed in the wild. There was no evidence for null alleles or for cross-reacting genetic variants. For each individual rabbit we determined the IgG genotype, at the *b* locus and at the *e* locus. Alleles of the *a* locus of the H-chain V region were also analyzed. In this paper we will focus on the nonrandom associations between alleles of the H- and L-chain C regions.

No b6 rabbits were found, and the *b9* allele was present in only 9 out of 16 populations with a mean frequency of 0.05. We can, therefore, treat the *b* locus as a diallelic locus. b9 and b4 have antigenic determinants in common, which distinguish them from b5 and b6 (12). The standardized interlocality variance in frequency (F_{ST}) (16) of b5 appeared to be lower than that of b4 (0.162) or b9 (0.210) (Table 1). For these reasons we will present part of the data by merging b9 together with b4 (17). "B" stands for the non-*b5* allele and "b" for the *b5* allele. Similarly "E" stands for *e15* and "e" for *e14*. Data analyses including the three *b*-locus alleles are shown in Table 1.

Linkage Disequilibrium Between the H- and L-Chain C-Region Loci. At the zygotic level, the *b5* allele of the L chain was associated with the *e14* allele of the H chain more often than expected: homozygous *b5* rabbits were of the e14 allotype almost twice more often than rabbits that were not b5. The nonrandom association of alleles of different loci is referred to as linkage disequilibrium (LD). It is characterized by a coefficient D, which measures the deviation of the observed gametic frequency from the frequency expected by random distribution of gametes. The Hardy–Weinberg expectation of the gametic frequency $f(b_ie_j)$ is the product of the allele frequencies $f(b_i)$ and $f(e_j)$. Therefore,

$$D(b_ie_j) = f(b_ie_j) - f(b_i)f(e_j).$$

For k alleles b_i and m alleles e_j, a standardized expression of D is given by the correlation coefficient R, such that (21, 22)

$$R^2(b_ie_j) = \sum_{i=1}^{k}\sum_{j=1}^{m}\frac{D^2(b_ie_j)}{f(b_i)f(e_j)}$$

The null hypothesis ($D = 0$) can be tested by $\chi^2 = 2NR^2$ [degrees of freedom (df) = $(k - 1)(m - 1)$], where $2N$ is the number of gametes sampled (17, 22).

To facilitate the comparison with a classical study on LD between independently segregating characters (i.e., chromosomal translocations), we have analyzed our data according to Lewontin and White (23) and Turner (24) as well as Wright (16). Since the loci are unlinked, the gametic types (*BE*, *Be*, *bE*, and *be*) cannot be distinguished in double-heterozygous animals and $D(be)$ is estimated by supposing that each type is equally likely in (*Bb*/*Ee*) rabbits. This leads in fact to the underestimation of the absolute value of D and to the underscoring of its significance (25).

Values of $D(be)$ were determined for all populations (Table 2). On one site (population 9), subareas (9 a–d and -r) were defined prior to data collection. The LD coefficients had the same sign at most localities (Table 2). Although none of the individual values were significant, together they strongly indicate that both loci are correlated. The weighted mean of $D_i(be)$ over all localities equals

$$D_S(be) = \sum_i (N_i/N)D_i(be) = 0.00873.$$

The value 0.00873 is actually very large for unlinked alleles

Table 1. Frequency variances, correlations, and linkage disequilibrium coefficients of H- and L-chain alleles in different areas

Allele	f	F_{ST}	$r(b_i,e14)$	$\mathrm{cov}(b_i,e14)$	$D_S(b_i,e14)$	$D_T(b_i,e14)$	$2NR_1^2$	$2NR_2^2$
				Australia ($2N$ = 1828, 9 localities)				
b5	0.27	0.015	0.66	0.00115	0.00684	0.00799	3.7	8
b4	0.71	0.014	−0.61	−0.00104	−0.00394	−0.00498	1.4	
b9	0.02	0.010	−0.22	−0.00010	−0.00290	−0.00300	5.1	
e14	0.197	0.008						
				Great Britain ($2N$ = 604, 4 localities)				
b5	0.23	0.284	0.93	0.02289	0.01269	0.03558	26.5	36
b4	0.62	0.767	−0.98	−0.04509	−0.00090	−0.04599	33.6	
b9	0.14	0.356	0.97	0.02220	−0.01180	0.01040	3.3	
e14	0.204	0.130						
				Continental Europe ($2N$ = 646, 3 localities)				
b5	0.37	0.108	0.34	0.00303	0.01041	0.01344	2.9	4.5
b4	0.57	0.071	−0.67	−0.00489	−0.01156	−0.01645	4.2	
b9	0.05	0.087	0.54	0.00187	0.00115	0.00302	0.8	
e14	0.218	0.040						
				Total ($2N$ = 3078, 16 localities)				
b5	0.282	0.079	0.72	0.00615	0.00873	0.01489	20.9	23
b4	0.667	0.162	−0.86	−0.01095	−0.00494	−0.01589	21.6	
b9	0.051	0.210	0.71	0.00479	−0.00379	0.00100	0.4	
e14	0.203	0.032						

f is the total gene frequency per area; F_{ST} estimates Wright's standardized interlocality variances in gene frequency (16) and measures the degree of genetic differentiation among populations from the area for the considered allele. F_{ST} tends to be larger at the *b* locus than at the *e* locus. The correlation coefficient $r(b_i,e14)$ equals $\mathrm{cov}(b_i,e14)/\sqrt{v(b_i)v(e14)}$, where cov and v are the biased estimates of the covariances and variances in allele frequencies. $2NR_1^2$ ($<\chi_1^2$) estimates the significance of the total LD D_T for two *b*-locus alleles, $2NR_2^2$ ($<\chi_2^2$) for three *b*-locus alleles. In each area the b5 and e14 frequencies are positively correlated, and within populations these alleles are found together in one individual more often than expected.

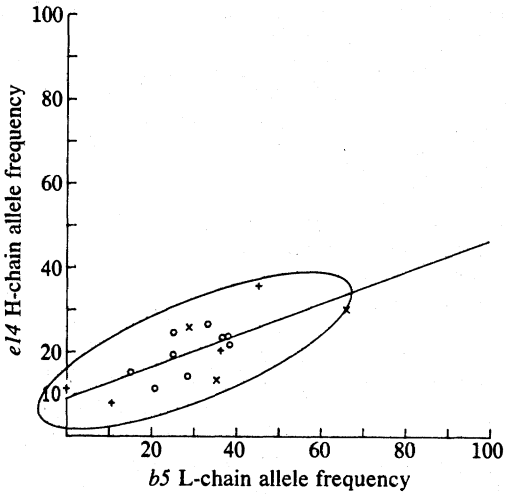

FIG. 1. Interlocality correlation and 95% equi-probability ellipse of the *b*-locus (*b5*) and *e*-locus (*e14*) allele frequencies. The correlation coefficient is $r = 0.72$ ($t = 9.9$, df = 14, $P < 10^{-5}$). The eccentricity of the ellipse (40) suggests that the population frequencies at both loci do not vary independently. Within each population we find nonrandom associations of alleles that are consistent with the interpopulation correlation in frequencies (Table 2). The shallowness of the slope of the linear regression has to do with the difference in frequency variances between both loci. ○, Australia; +, Great Britain; ×, continental Europe.

and would, if caused by epistatic interactions in fitness, indicate very strong selection (see *Discussion*).

On the total sample, the total association coefficient $D_T(be)$ equals

$$D_T(be) = f_T(be) - f_T(b)f_T(e) = 0.01489,$$
$$\chi^2_1 > 2NR^2_T = 21.$$

It is easily shown that (26)

$$D_T(be) = D_S(be) + \text{cov}(be),$$

where cov(*be*) is the biased estimate of interlocality covariance in gene frequencies, weighted by sample size. The correlation between *b*- and *e*-locus frequencies is visualized in Fig. 1. Because cov(*be*) = 0.00615 (Table 1), $D_T(be)$ reaches about 10% of its theoretical maximum value [i.e., if $f(eB) = 0$]. Values for the different areas and for the other *b*-locus alleles are also shown (Table 1). The crude data shown in Table 2 allow the calculation of variance components of LD as proposed by Ohta (28) for subdivided populations. With three *b*-locus alleles we obtained for the moment of variance of D_i, $D^2_{IS} = 0.000664$, and for the variance of the correlation, $D^2_{ST} = 0.049277$.

Viability Matrix. At each locality the observed numbers of zygotes were determined for each of the nine phenotypic classes, and the "expected" zygote frequencies were calculated from the relative allele frequencies (23) or from the gametic frequencies (24). These values and their ratio are shown in Table 3 for the one population where a majority of the animals was analyzed (population 9). These ratios might give a first indication on the relative viabilities associated

Table 2. Gametic associations, gene frequencies, and zygote numbers of L-chain (*b*-locus) and H-chain (*e*-locus) allotypes

		Number of zygotes observed with genotype*											
i	$2N_i$†	*BB/EE*	*Bb/EE*	*bb/EE*	*BB/Ee*	*Bb/Ee*	*bb/Ee*	*BB/ee*	*Bb/ee*	*bb/ee*	$f_i(b)$	$f_i(e)$	$D_i(b,e)$ × 1000
						Australia							
1	166	22	19	6	13	12	8	1	2	0	0.367	0.235	+10.05
2	142	21	18	0	16	10	3	2	0	1	0.253	0.246	+7.93
3	14	3	3	0	0	0	0	0	1	0	0.286	0.143	+30.61
4	46	12	5	0	4	0	1	1	0	0	0.152	0.152	−1.42
5	30	4	3	0	2	5	1	0	0	0	0.333	0.267	+27.78
6	158	37	22	2	11	7	0	0	0	0	0.209	0.114	−1.64
7	42	5	5	2	4	3	1	0	0	1	0.381	0.238	+16.44
8	78	9	13	1	4	8	3	0	1	0	0.385	0.218	+18.74
9‡	1152	211	157	11	86	72	12	13	13	1	0.252	0.194	+5.74
9a	256	33	22	0	33	25	5	6	4	0	0.238	0.324	+6.73
9b	190	35	27	3	6	10	1	5	7	1	0.284	0.226	+14.67
9c	260	60	36	3	18	10	3	0	0	0	0.223	0.119	+4.17
9d	256	49	37	2	18	20	1	1	0	0	0.246	0.160	+3.56
9r	190	34	35	3	11	7	2	1	2	0	0.284	0.137	+0.58
						Great Britain							
10	178	62	13	0	8	6	0	0	0	0	0.107	0.079	+8.46
11	44	7	6	1	1	5	1	0	1	0	0.364	0.204	+27.89
12	150	58	0	0	17	0	0	0	0	0	0	0.113	0
13	232	18	24	8	16	21	12	4	6	7	0.453	0.358	+21.27
						Continental Europe							
14	246	37	47	9	11	14	2	0	2	1	0.354	0.134	+5.40
15	106	6	12	11	1	7	8	0	3	5	0.660	0.302	+31.77
16	294	45	33	6	20	28	2	6	6	1	0.289	0.259	+6.90
Total	3078	557	380	57	214	198	54	27	35	17	0.282	0.203	+14.89

The zygote numbers are presented by collapsing *b4* and *b9* to "*B*," the allele of "*b*" (*b5*) . $f_i(b)$ and $f_i(e)$ are the relative gene frequencies of *b5* and of e14. $D_i(b,e)$ is the LD coefficient between these alleles at locality *i*. The expectation that on the average $D_i(b,e) = 0$ is clearly not fulfilled.

*($b = b5$; $B = b4 + b9$; $e = e14$; $E = e15$.

†Number of gametes in locality *i*.

‡Localities 9a–9r are regions within locality 9.

Table 3. Estimation of viabilities of combined *b*- and *e*-locus genotypes in population 9

Phenotype	Number observed, O	Number expected, E^a	Ratio O/E^a	Number expected, E^b	Ratio O/E^b
B E	211	211.5	1.00	215.3	0.98
B Ee	86	97.1	0.89	94.4	0.91
B e	13	14.2	0.92	13.1	0.99
BbE	157	141.6	**1.11**	139.1	**1.13**
BbEe	72	65.3	**1.10**	67.2	**1.07**
Bbe	13	9.4	**1.38**	10.1	**1.29**
b E	11	24.1	**0.46**	22.8	**0.48**
b Ee	12	11.1	1.08	12.0	1.00
b e	1	1.6	0.63	2.0	0.50

The expected numbers (E^a and E^b) are the sums over expected numbers within subdivisions (576 zygotes, five subdivisions), following either Lewontin and White (23) "E^a" or Turner (24) "E^b." O, observed numbers. The values could indicate that mortality is higher in rabbits doubly homozygous for the *b5* and *el5* alleles, whereas heterozygous *b5* rabbits have an increased viability (relevant values are indicated by bold numbers).

with the different genotypes, in case the observed LD was caused by selection. Most striking was the shortage of double homozygous *b5/e15* rabbits: as can be calculated from the data in Table 2, in each population fewer bE phenotypes (i.e., *b5b5/e15e15*) were observed than expected, whereas there was a rather systematic excess of heterozygous *b5* rabbits (*Bb*).

Consistent LD was not found (data not shown) when we analyzed the associations between *b* locus and *a* locus (H-chain V region), which is closely linked to the *e* locus (1, 2). This suggests that the LD observed between the C-region alleles cannot be explained by selection on the V regions.

DISCUSSION

The consistency of the correlations within populations [$D_i(be)$] and between populations [cov(*be*)] is not unexpected, since LD and correlations in gene frequencies among populations are different expressions of the same fact, and one can be explained by the other. The empirical observations of both phenomena are, however, independent. Consistent LD between alleles of the Ig C regions was first observed in Australia and was subsequently confirmed in Great Britain and continental Europe. The significance of the associations between the H- and L-chain alleles is, therefore, very high as is also indicated by the χ^2 tests on the total sample (Table 1).

LD can result from stochastic effects, such as migrations, historical sampling, and population subdivision (21–26, 28), but consistent LD was so far not reported for unlinked protein loci. This is not surprising since LD between unlinked loci should decay very rapidly. Even between linked loci, consistent LD is generally not observed (29). The systematic correlation of defined alleles of the H- and L-chain C regions, within and between populations on different continents, each with a different history of rabbit colonization, is therefore interesting in its own right.

On the other hand, consistent LD has been predicted in situations where different genotypes have different viabilities (21, 28, 30). As pathogens can interfere with the immune system of the host by interacting with the Ig C regions (5), fitness differences among Ig allotypes could obviously exist as an adaptive or nonadaptive consequence of such parasite–host interactions and could explain the nonrandom allele associations. The observed systematic shortage of (*b5b5/e15e15*) rabbits could indeed mean that rabbits with this genotype have a reduced chance to survive. If this was the case the polymorphisms should be maintained by (double) heterozygote advantage. The data do indeed indicate a rather strong overdominance of alleles at least at the *b* locus, independent of the associated H-chain alleles.

The sample sizes are too limited to allow the estimation of the coefficients of overdominance and epistasis, but it is clear that the total selection force involved could be extremely strong. According to Hastings (31) a value of $D = 0.009$ between unlinked alleles requires a selection factor of 0.045. This would mean that 9% of mortality is correlated to less-favorable combinations of Ig allotypes, which is exceedingly high for only two loci. This is, however, less implausible if one considers the very dramatic impact of myxomatosis on rabbit populations: this viral disease had an initial mortality rate of more than 99% when it was introduced some 30 years ago and might have killed between 80% and 90% of all wild rabbits. At present, myxomatosis still acts as a major mortality factor in the areas under study (18, 19, 32).

In the context of the population genetics data here presented, it is interesting that the mode and rate of mutant acceptance at the genetic loci concerned (see the Introduction) differ from what is generally observed in protein evolution. The *b4* and *b5* genomic sequences differ in their coding regions (330 base pairs) by 39 base-pair substitutions at amino acid replacement sites for only four at synonymous sites, while 96% of the base pairs of the 3′-untranslated region are conserved (7–9). A somewhat similar situation exists for Ig C-region genes in other species (33, 34). Johnson *et al.* (35) have suggested that overdominance, by preventing the random fixation of alleles, has allowed the accumulation of diversity between the human Ig H-chain haplotypes. The proposed mechanisms could also explain the extreme divergence between the *b* locus alleles but not the relatively low acceptance of silent mutations. For reasons outlined in the neutral theory (ref. 36; for a detailed discussion, see ref. 37) the high ratios of replacement versus silent nucleotide substitutions observed between the κ L-chain alleles in rat and rabbit do in fact provide strong indications for positive selection favoring structural change and diversity between Ig alleles (9, ¶). Overdominance and epistasis between alleles of the H- and L-chain C regions might, therefore, have played an active role in the evolution of polymorphism at these loci.

A related question is whether epistatic fitness interactions could also account for differences in "fixation index" (F_{ST}) between interacting alleles and thus for differences in rate of mutant substitution. It is in this context interesting that the two *e* locus markers were observed in a primitive leporid species (the Mexican volcano rabbit *Romerolagus diazi*) at gene frequencies $f(e14) = 0.24$ and $f(e15) = 0.76$ (15) while no interspecies overlap of *b* locus alleles was observed (11–15).

On a phylogenetic time scale, epistasis is also expected to favor linkage between the interacting loci (38). For H- and L-chain components of the antibody, however, the necessity for maximizing the diversity at the antigen binding regions at the individual and at the population levels might have prevented the formation of such a linkage, as this should restrain the recombination between H- and L-chain V-region genes. The absence of linkage might also be related to the phenomenon of allelic exclusion (39).

Direct evidence for selection can only be obtained from age-specific fecundity and mortality tables, and, especially with regard to myxomatosis, more direct approaches and experiments will be required before the observed LD can be

¶van der Loo, W., Third International Congress of Systematic and Evolutionary Biology, July 4–10, 1985, Brighton, U.K., 108 (abstr.).

considered a measure rather than an indicator of selection. At this stage, the point is that a number of independent and quite exceptional observations on the nature and on the behavior of the rabbit Ig allotypes could be explained if we assume that the H- and the L-chain polymorphisms are not selectively neutral and that fitness can depend upon the combined genotype of the antibody. The systematic nonrandom associations and the degree of structural divergence between Ig alleles could thus be, respectively, short-term and long-term effects of selection forces that have modulated evolutionary change and phenotypic variation of the Ig C regions within populations. In our opinion, the particular situation created in the European rabbit by the introduction of myxoma virus offers a rather unique opportunity to study the nature of these selection forces and the way they affect genetic variation and evolutionary change at the molecular interface between parasite and host.

We thank John Ross, Peter Garson, and Philip Kerremans for the collection of part of the rabbit blood samples; Cecile Casterman for the characterization of the antisera; and Maria Vanderveken for technical assistance. Jan Naessens and Willy Verheulpen were helpful with computer analyses. We are also much indebted to Albert Jacquard, Richard C. Lewontin, and Domien Roggen for their encouragement and for helpful discussions and to Richard K. Koehn for having read and corrected a first version of the manuscript. This work was in part supported by the Belgian National Science Foundation (N.F.W.O.). W.v.d.L. is a research associate of this Foundation.

1. Oudin, J. (1960) *Exp. Med.* **112,** 107–124.
2. Mage, R., Lieberman, R., Potter, M. & Terry, W. D. (1973) in *The Antigens*, ed. Sela, M. (Academic, New York), Vol. 1, pp. 299–376.
3. Hood, L., Campbell, J. H. & Elgin, S. C. R. (1980) *Annu. Rev. Genet.* **9,** 305–353.
4. Ohta, T. (1982) *Proc. Natl. Acad. Sci. USA* **79,** 3251–3254.
5. Hamers, R., van der Loo, W. & De Baetselier, P. in *The Ruminant Immune System in Health and Disease*, ed. Morrison, I. (Cambridge Univ. Press, Cambridge, U.K.), in press.
6. Farnsworth, V., Goodfleesh, R. M., Rodkey, S. & Hood, L. (1976) *Proc. Natl. Acad. Sci. USA* **73,** 1293–1296.
7. Bernstein, K. E., Skurla, R. M., Jr. & Mage, R. (1983) *Nucleic Acids Res.* **11,** 7205–7214.
8. Emorine, L., Sogn, J. A., Tring, F. D., Kindt, T. J. & Max, E. E. (1984) *Proc. Natl. Acad. Sci. USA* **81,** 1789–1793.
9. Matthyssens, G., Borremans, B., Hamers, R. & van der Loo, W. (1985) in *Protides of the Biological Fluids*, 33rd Colloquium, Brugge, ed. Peeters, H. (Pergamon, Oxford), pp. 123–126.
10. Appella, E., Chersi, A., Mage, R. G. & Dubiski, S. (1971) *Proc. Natl. Acad. Sci. USA* **68,** 1341–1345.
11. Landucci-Tosi, S., Mage, R. C. & Tosi, R. (1976) *J. Immunol.* **117,** 679–685.
12. van der Loo, W. (1975) *Allelic Polymorphism and Concomitant Evolution of Co-functional Genes of the Lagomorph Immunoglobulins* (Vrije Univ., Brussels, Belgium).
13. van der Loo, W., Hamers-Casterman, C. & Hamers, R. (1976) *Arch. Int. Physiol. Biochim.* **84,** 192–193.
14. Cazenave, P. A., Brezin, C., Puget, A. & Mandy, W. (1979) *Immunogenetics* **4,** 489–492.
15. van der Loo, W. & Hamers-Casterman, C. (1981) in *Proceedings of the World Lagomorph Conference Guelph, Ontario 1979*, eds. Myers, K. & Mac Innes, C. D. (Univ. of Guelph, Guelph, ON), pp. 17–27.
16. Wright, S. (1978) *Evolution and Genetics of Populations* (Univ. of Chicago Press, Chicago), Vol. 4.
17. Brown, A. M. D. (1975) *Theor. Popul. Biol.* **8,** 184–201.
18. Fenner, F. & Ratcliffe, F. N. (1965) *Myxomatosis* (Cambridge Univ. Press, Cambridge, U.K.).
19. Ross, J. & Tittensor, A. M. (1981) in *Proceedings of the World Lagomorph Conference Guelph, Ontario 1979*, eds. Myers, K. & Mac Innes, C. D. (Univ. of Guelph, Guelph, ON), pp. 830–833.
20. Hamers-Casterman, C., Wittouck, E., Florent, G., van der Loo, W., De Baetselier, P. & Hamers, R. (1977) *J. Immunogenet.* **4,** 301–314.
21. Cavalli-Sforza, L. L. & Bodmer, W. F. (1971) *The Genetics of Human Populations* (Freeman, San Francisco).
22. Weir, B. S. & Cockerham, C. C. (1978) *Genetics* **88,** 633–642.
23. Lewontin, R. C. & White, M. J. D. (1960) *Evolution* **14,** 116–119.
24. Turner, J. (1972) *Evolution* **26,** 334–343.
25. Cockerham, C. C. & Weir, B. S. (1977) *Genet. Res. Commun.* **30,** 121–147.
26. Nei, M. & Li, W. (1973) *Genetics* **75,** 213–219.
27. Hauspie, R., Defrise-Gussenhoven, E. & Susanne, C. (1976) *Bull. Soc. R. Belge Anthrop. Préhist.* **87,** 77–88.
28. Ohta, T. (1982) *Proc. Natl. Acad. Sci. USA* **79,** 1940–1944.
29. Mukai, T., Tachida, H. & Ichonose, M. (1980) *Proc. Natl. Acad. Sci. USA* **77,** 4857–4860.
30. Lewontin, R. C. (1974) *The Genetic Basis of Molecular Change* (Columbia Univ. Press, New York).
31. Hastings, A. (1981) *Genetics* **98,** 659–668.
32. Anderson, R. M. & May, R. M. (1982) *Parasitology* **85,** 411–426.
33. Scheppard, H. W. & Gutman, G. A. (1981) *Proc. Natl. Acad. Sci. USA* **78,** 7064–7068.
34. Schreier, P. H., Bothwell, A. L. M., Mueller-Hill, B. & Baltimore, D. (1981) *Proc. Natl. Acad. Sci. USA* **78,** 4495–4499.
35. Johnson, J. M., de Lange, G. & Cavalli-Sforza, L. L. (1986) *Am. J. Hum. Genet.* **38,** 617–640.
36. Kimura, M. (1968) *Nature (London)* **217,** 624–626.
37. Miyata, T. (1982) in *Molecular Evolution, Protein Polymorphism and the Neutral Theory*, ed. Kimura, M. (Japan Sci. Soc. Press., Tokyo).
38. Kimura, M. (1956) *Evolution* **14,** 458–472.
39. Ritchie, K. A., Brinster, R. L. & Storb, U. (1984) *Nature (London)* **312,** 517–520.