Greek temples, tropical kine and recombination load

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Abstract

New breeds typically arise from the cross of two or more existing breeds, often chosen to complement each other. For about 100 years, efforts have been made in the tropics to produce a milking breed combining zebu tropical adaptation with taurus production ability. All attempts have failed, although the F_1 in nearly all instances has been superior. The Greek Temple Model (GTM) is a graphical representation of crossbreeding theory which completely accounts for intralocus genetic effects, but not epistatic effects. All crosses except the F_1 may lose merit due to destruction by recombination at meiosis of favorable epistatic combinations built up during reproductive isolation. Data from a meta analysis of 80 reports of taurus-indigenous breed crosses in the tropics were used to fit the GTM and to estimate recombination load. Data for lactation length and calving interval fit the GTM ($P \approx 0.3$) indicating that epistasis was not an important source of genetic variance in the crosses; however, data for lactational milk yield, age at first calf and annual milk yield did not. The GTM was extended to include recombination load under a two-locus model, and satisfactory agreement was found for the remaining traits ($0.6 < P < 0.9$). Minimum χ^2 estimates of recombination load for lactational milk yield and annual milk yield were -277 ± 55 kg and -345 ± 56 kg, respectively. Three kinds of loads were necessary to account for breed cross means for age at first calving. Heifers having an intact taurus but impaired zebu complex were predicted to calve about 3.5 ± 1.5 months earlier while those having the alternative combination were predicted to calve 2.8 ± 8 months later. Heifers lacking either ancestral complex calved 8.9 ± 7.2 months later. Plausibility of the results was discussed in light of recent evidence suggesting at least a quarter-million-year separation of the two races of cattle. Long periods of time would be necessary for interaction systems to have evolved differently and produce load upon crossing. It is concluded that the 100-year effort to breed a tropically adapted dairy cow from a zebu×taurus base failed, not because of lack of effort or tenacity or faulty methodology, but because recombination between incompatible genetic interaction systems caused too great a load on the crossbred generations. In vitro fertilization and allied technologies are suggested as a way to exploit the obvious advantage of the F_1 and to circumvent genetic load. © 2001 Elsevier Science B.V. All rights reserved.

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1. Introduction

For most of the present century, crosses have been made in the tropics between temperate dairy breeds and adapted (usually zebu) local breeds to establish base populations from which selection could be used

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to create tropically adapted and productive dairy breeds (Cunningham and Syrstad, 1987; McDowell, 1985; Rege, 1998). None of the scores of attempts has succeeded, at least to the extent that one of the 'breeds' released from these research efforts is widely used as a pure breed in tropical areas (McDowell, 1985; Maule, 1990; Payne and Hodges, 1997). In nearly all cases, the F_1 generation was superior, exhibiting both production ability and adaptation, but generations subsequent to the F_1 were usually disappointing (Cunningham and Syrstad, 1987). In particular, the F_2 generation fails to meet expectation (Syrstad, 1989). He gave a cogent argument for a recombination load and breakup of epistatic complexes in the F_2 as being causative, but no statistical test of this hypothesis was made.

The absence of a new dairy breed to have emerged from all the crossbreeding work with cattle suggests there may be a fundamental flaw with the material or the approach. It is unlikely that the approach has been *wrong*; nearly all breeds, cattle or otherwise, began from some crossbred base. Furthermore, selection for a trait like milk production is not too difficult. In some instances, the selection scheme employed may have been inefficient, but in no case has the expectation of genetic change been zero. This leaves suspicion that the material may be flawed. The consistent failure may be a signal of a genetic load resulting from recombination at meiosis. Herein we develop methodology and test this hypothesis.

2. Materials and methods

2.1. Data

Rege (1998) assembled and analyzed data from 80 reports on crosses between temperate dairy breeds and indigenous cattle in the tropics. He reported least-square means for various gradations of taurus inheritance for lactational milk yield (MY), lactation length (LL), age at first calf (AFC), calving interval (CI) and annual milk yield (AMY) calculated as $365 \cdot MY/CI$. These means and their standard errors were data for the present work. Data from the F4 and crosses higher than 7/8 were not included in the present analysis because of incompleteness and/or relatively large standard errors.

2.2. Theory

Cunningham (1987) developed a geometrical representation of crossbreeding theory known as the Greek Temple Model (GTM) since it often resembles the front elevation of a classic Greek temple. The GTM was developed primarily as a didactic tool and is an innovative way to visualize crossbreeding theory. Theory on which the GTM is based (Dickerson, 1972; Willham and Pollak, 1985) fully accounts for additive effects and dominance deviations at independent loci and extends in a natural way to between-breed additive differences and heterosis resulting from dominance and/or overdominance. Extensions of the GTM to between-locus interactions are frustrated by the large number of possible parameters (Cockerham, 1954; Weir and Cockerham, 1977). In constructing the GTM, a base (or foundation) is employed on which the proportion taurus breeding from 0 to 1 is displayed (Fig. 1). Heights of the sides (or walls) are proportional to the means (P_1, P_2) of the two purebreds. A roof is formed by connecting the F_1 mean to the parental means. Under
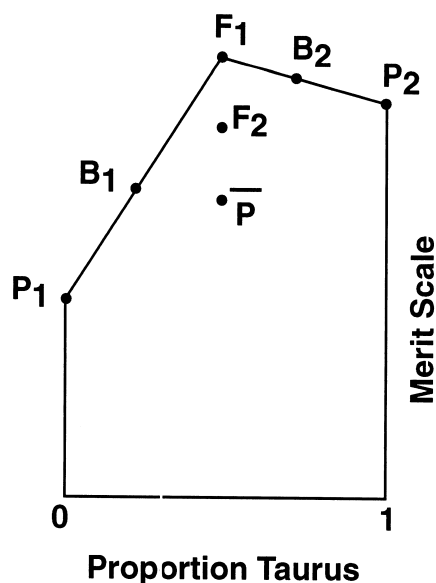

Fig. 1. Cunningham's GTM. In absence of epistasis, expected values of all intergrades between the two parental breeds (P_1 and P_2) lie on the roof the peak of which is determined by the F_1. F_2 and subsequent inter se generations have expected value intermediate between the F_1 and parental mean.

assumption that epistasis is nil, expected values for the performance of all crosses (except inter se generations) lie on the roof. Inter se crosses have expected value at the midpoint between the 'roof' and the straight line connecting the parents; this loss of performance arises from loss of heterozygosity in inter se matings.

If epistasis contributes to performance, and if meiosis destroys favorable combinations, then the observed value of a cross should be less (with positive heterosis) than its GTM expectation. In writing of departures of F_2 and backcross generations from their expected values, Wright (1968) wrote: ''A significant deviation from any of these relations in the absence of differential viability demonstrates the existence of interaction.'' Note that since purebreds and their F_1 are formed from gametes in which there was no possibility of between-genome recombination, the means of these groups can be used to construct the GTM without bias due to recombination load.

Recombination load results from the break-up of ancestral coadapted gene complexes during meiosis and is a manifestation of Mendel's Law of Independent Assortment. It will be convenient to suppose two long separated breeds where 'long' implies the evolutionary time needed for interaction systems to become disparate. Let the 'Latin' breed have genotype aabb and the 'Greek' breed have genotype ααββ. Alleles a and b may be distinct from α and β in terms of sequence, but if they are functionally equivalent, no load occurs upon recombination. Purebreds and their F_1 experience no load, but individuals of all other crosses have a probability of recombination load. Inspection of the Punnett square for a dihybrid cross (Table 1) suggests that

the progeny can be classified in six ways. There are 'recovered purebreds', of which there are two, and a 'recovered F_1'; none of these experience load. The fourth type has an intact Latin complex, but an incomplete Greek complex (aabβ or aαbb). The fifth type has an intact Greek complex, but an incomplete Latin complex (ααbβ and aαββ). The last type are those having neither complex intact (aaββ and ααbb). Let the loads corresponding to the last three types be defined as recombination loads of the first (R_1), second (R_2) and third (R_3) kinds, respectively.

2.3. Analysis

Cunningham's (1987) GTM furnishes expected values for hybrid groups under a theory which does not include between locus interaction, and Rege (1998) gives data collected over a wide range of environments and of samples of taurus and zebu parents and their hybrids. Respectively, these constitute the expected and observed values for the χ^2 analysis outlined below. Fitting was done in a stepwise fashion starting with the simplest model and proceeding to the more complex until an adequate fit had been found in accordance with Occam's Razor. Thus I first tested the fit of the GTM assuming no recombination load ($R_i = 0$) to the data using the method of minimum χ^2 (Fisher, 1970; Kendall and Stuart, 1973) by calculating

$$\chi^2 = ...(Observed_I + ...a_jR_j - ExpectedI)^2 / (Variance\ of\ the\ Observed)_i \qquad (1)$$

where the first summation runs across the i classes (breed groups). In Eq. (1) a_j is the frequency of individuals in class i expected to incur load R_j. In assigning the a_j (Table 2) I assumed that 5/8 and 3/8 blood groups were formed from the cross of the F_1 and the appropriate backcross and that except for the F_2 and F_3, none of the crosses contained data from inter se matings. The statistic defined in Eq. (1) is distributed as χ^2 with degrees of freedom equal to the number of breed groups not used to construct the GTM or to estimate the parameters (R_i) of the extended GTM. With assumption of normal distribution of the observed values, the minimum χ^2 estimates of the R_j are maximum likelihood (Meyer, 1975).

Table 1
Punnett square showing kinds of recombination load (R_i) in progeny of a dihybrid cross[a]

Ova	Sperm			
	ab	aβ	αb	αβ
ab	aabb (none)	aabβ (R_1)	aαbb (R_1)	aαbβ (none)
aβ	aabβ (R_1)	aaββ (R_3)	aαbβ (none)	aαββ (R_2)
αb	aαbb (R_1)	aαbβ (none)	ααbb (R_3)	ααbβ (R_2)
αβ	aαbβ (none)	aαββ (R_2)	ααbβ (R_2)	ααββ (none)

[a] R_1 (R_2) results from lack of intact latin (greek) coadapted sequence; R_3 results from lack of both sequences.

Table 2
Relative abundance of load and no load genotypes in selected crosses between two lines (L and G) for two freely recombining loci

Proportion G[a]	Parents[b]	No load genotypes[c]			Genotypes subject to load[d]		
					aabβ aαbb	aαββ ααbβ	aaββ ααbb
		aabb	ααββ	aαbb			
0	L×L	1	0	0	0	0	0
1/8	L×BC_L	0.56	0	0.06	0.38	0	0
	Inter se	0.59	<0.01	0.05	0.33	0.01	0.02
1/4 (BC_L)	F_1×L	0.25	0	0.25	0.5	0	0
	Inter se	0.32	<0.01	0.14	0.42	0.05	0.07
3/8	F_1×BC_L	0.16	0	0.25	0.38	0.13	0.09
	L×BC_G	0.06	0	0.56	0.38	0	0
	3/8L×7/8L	0.05	0.01	0.47	0.30	0.12	0.04
	Inter se	0.15	0.02	0.22	0.37	0.13	0.11
1/2(F_1)	L×G	0	0	1	0	0	0
	Inter se	0.06	0.06	0.25	0.25	0.25	0.13

[a] Symmetry arguments suffice for proportion $G > 0.50$.

[b] L and G are pure lines; BC_i indicates a backcross to line i.

[c] Alleles a and b derive from the L line while α and β come from G.

[d] From left, loads incurred are of the 1st, 2nd and 3rd kinds, respectively. Under Castle, Hardy, Weinberg conditions the alternative genotypes within column occur with equal frequency.

It could occur that the loads of the three kinds were equal, so the next step in fitting strategy was to fit Eq. (1) using a single R and letting a be the sum of the frequencies of the last three types. If that did not fit I assumed 2 Rs, pooling the loads for the 4th and 5th types and separating the load for the 6th type. Finally, if that did not fit I assumed three distinct loads.

Frequencies of individuals experiencing the various kinds of loads depend upon the number of functionally distinct loci involved in the interaction system. Thus the logical extension of the above fitting/estimating process would be to assume more than two loci. Since that was not necessary here and it is a straightforward extension of the two-locus case, that theory will not be further considered. Further, it should be understood that the present analysis gives no information on the total number of loci influencing a trait.

3. Results and discussion

Breed–group means from Rege (1998) are shown (as solid dots) in Fig. 2. The F_3 and 1/8 groups had large standard errors (shown as hemistandard error bars) as a consequence of their rare inclusion, and few observations if included, in the 80 experiments. Since the χ^2 statistic contains the variance in the denominator, groups with large standard errors have little influence relative to those with smaller standard errors. All reports would have included data from the zero taurus and F_1 groups and most would have had data from the pure taurus. As a result, these groups have relatively small standard errors, and this is fortuitous since construction of the GTM as done here assumes that the means of these groups are known with negligible error.

The GTM expectation of the F_2 and F_3 is shown as a diamond. For the other crosses, the GTM expectation lies on the 'roof.' For all traits except calving interval, the F_2 mean differs from GTM expectation by more than two standard errors, and it differs in the direction of poorer performance — less milk production, shorter lactations, older age at onset of production and longer intervals between production cycles. Syrstad's (1989) review concentrated on the performance and adaptation of this cross, and he reached similar conclusions. The overall superiority of the F_1 is evident in these data, and this superiority was no doubt a prime motivation for all of the breeding work with zebu×taurus crosses of the last
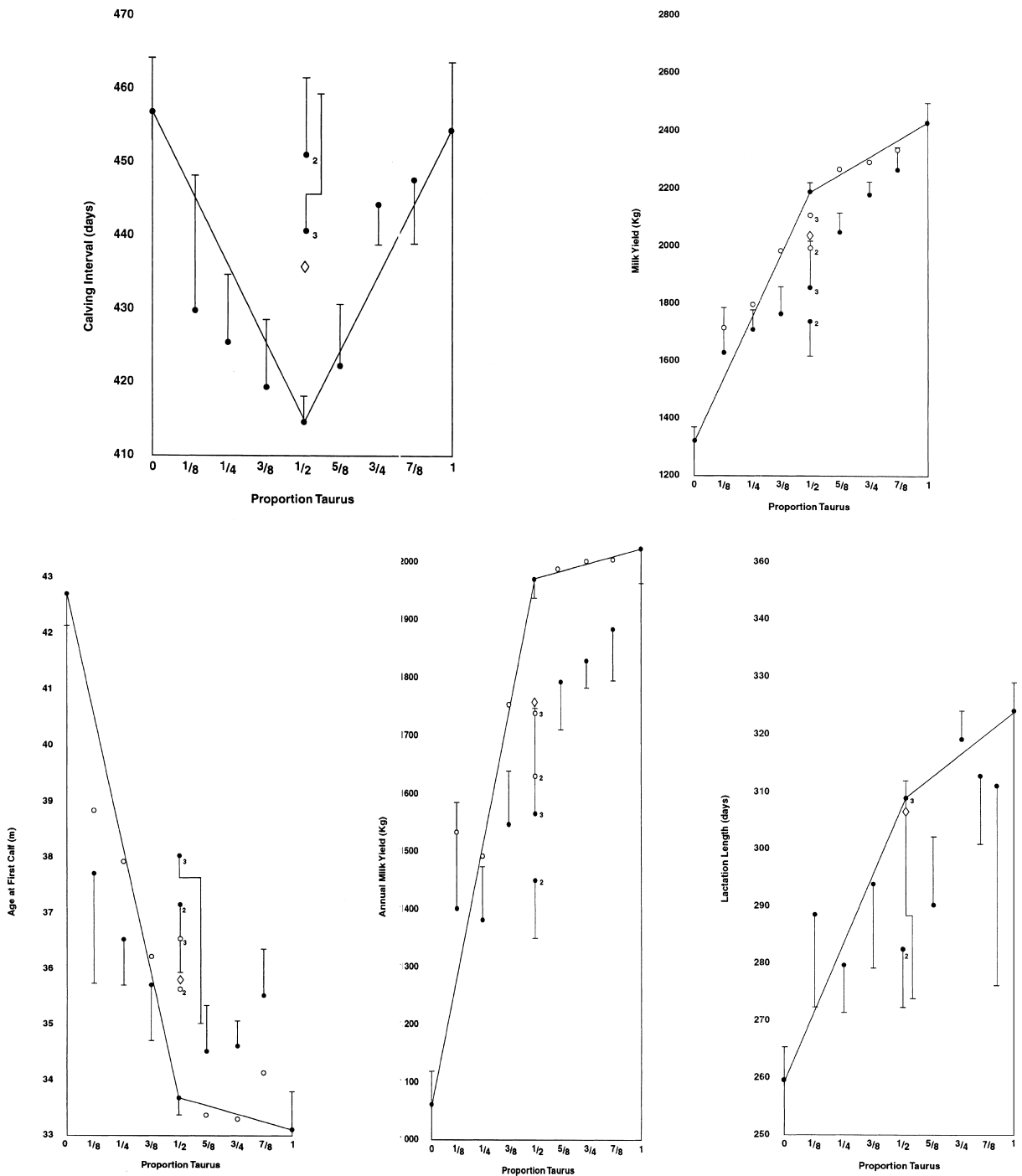
Fig. 2. Greek Temple Model fit to Rege's data. Solid dots with hemistandard error bars are observed values. Subscripts indicate filial generation. Open circles are observed values adjusted for estimated recombination load. Open diamond gives expected value to the F_2 and F_3.

century. Open circles in Fig. 2 are fitted values assuming recombination load(s). None appear in the panels for lactation length and calving interval as the GTM fit these data, $P \cong 0.3$ (Table 3). There was, thus, no evidence for recombination load causing variability in these two traits. This finding does not imply that epistasis is unimportant for these traits as it could be that an ancestral genetic interaction system has been conserved in both parental races.

Data for milk yield and annual milk yield did not fit the GTM ($P < 0.0001$). However, a single recombination load common to the three kinds of load gave an adequate fit for milk yield ($P \approx 0.6$) and annual milk yield ($P \approx 0.9$). Proportion of affected individuals depends upon specific cross (Table 2), but this analysis implies that 5/8 of an F_2 generation would have milk yields decreased by 277 ± 53 kg due to recombination load. Estimated recombination load for annual milk yield was 345 ± 56 kg. A somewhat more involved analysis was necessary for age at first calving (Table 4). Zebu cattle are well known for having an advanced age at first calving relative to taurus (Cunningham and Syrstad, 1987), and the data did not fit the GTM ($P < 0.005$). Assumption of a load common to the three kinds gave a significant reduction ($P < 0.05$) in the χ^2, but the model still failed to adequately describe the data ($P < 0.005$). Assumption that recombination load for an intact zebu complex but incomplete taurus complex was

equivalent to that for an intact taurus but incomplete zebu complex along with a recombination load of the third kind did not improve the fit ($P < 0.01$). A dramatic improvement in fit occurred with removal of the constraint that recombination loads of the first and second kinds were equal as the χ^2 dropped to 3.5 with a probability of about 0.6. Heifers with an intact zebu complex but incomplete taurus complex were delayed at first calving by 2.8 ± 0.8 months, while heifers with an intact taurus complex but incomplete zebu complex calved 3.5 ± 1.5 months earlier. Heifers having neither ancestral complex intact had an increased age at first calving of 8.9 ± 7.2 months. Delays in onset of production severely limit usefulness as a dairy cow. The estimate of recombination load of the third kind was particularly severe and fully 12% of an F_2 population experience such a load (Table 2).

Zebu and taurus breeds differ greatly in milk production and in age at puberty, the two traits for which recombination load was found. Milk production differences between the two taxa probably occurred relatively recently (certainly since domestication and most likely within the last 1000 years) compared to age at puberty. This may account for the increased apparent genetic complexity of age at first calf since there would have been much more time for genetic diversity to have evolved.

Mackinnon et al. (1996), from analysis of records

Table 3
Minimum χ^2 fits to Rege's data[a]

Statistics	LL	CI	MY	AMY
χ^2 ($R_i = 0$)	9.1	9.6	32.6	40.2
Probability	$\cong 0.3$, 8 d.f.	$\cong 0.3$, 8 d.f.	< 0.0001	< 0.0001
χ^2 ($R_1 = R_2 = R_3$)	N.a.	N.a.	5.6	2.9
Probability	N.a.	N.a.	≈ 0.6, 7 d.f.	≈ 0.9, 7 d.f.
Recombination load	0	0	277 ± 53	345 ± 56

[a] LL, lactation length; CI, calving interval; MY, lactational milk yield (kg); AMY, annual milk yield (kg).

Table 4
Minimum χ^2 fits to age at first calf and estimated recombination loads (R_j)

Assumed load	χ^2	df	P	R_1	R_2	R_3
None	22.9	8	< 0.005	–	–	–
$1 = 2 = 3$	17.0	7	< 0.05	-1.4	–	–
$1 = 2$, 3	17.0	6	< 0.01	-1.4	-1.4	$-.72$
1, 2, 3	3.5	5	≈ 0.6	3.5 ± 1.5	$-2.8 \pm .8$	-8.9 ± 7.2

of a long term interbreeding population of Sahiwal (an Indian Zebu breed) and Ayrshire and Brown Swiss (both taurus breeds), claimed a positive recombination effect for milk yield. This is a singular result in the literature for taurus×zebu crosses and may be a sampling error; the authors noted "the estimates are unlikely to be reliable."

It is clear that all zebu×taurus crosses excepting the F_1 have recombination loads for milk production and the onset of production. This load usually increases in the first inter se generation but not thereafter under random mating. However, from the superiority of the F_1 it seems reasonable to assume that the more heterozygous individuals of a particular generation would be selected as parents; this could cause load beyond that expected under random mating. Gametes from heterozygotes are the most likely to carry genes that are not coadapted. Half of the F_1 gametes are of this sort. On the strength of the current evidence, the failure to obtain a tropically-adapted dairy breed from a taurus×zebu base during the last century of work was probably not due to lack of effort, improper design or lack of tenacity. Rather, the failure was due to a basic flaw in the genetic material. Specifically, that flaw is a recombination load due to the recombining of ancestral genomes.

Of course, milk production is not the sole measure of the utility of a particular group for dairying in the tropics. They should possess, in addition to capacity to produce milk, the ability to thrive. (I would like to use the words fit and fitness for thrive and thrift, but these words have well-established specific meanings in population genetics and are unavailable). An animal that is fit has a high reproductive rate relative to cohorts, but a thrifty animal is one particularly well-suited for some purpose which may or may not involve reproduction. The mule has great thrift, but its fitness is zero. In crosses between species in cattle (genus Bos), males are typically sterile while females are fertile (Haldane, 1922), so there is a genetic load and thus a loss of fitness. Hybridization in this genus is both an ancient and common practice and thrifty animals result. Marco Polo reported the practice of crossing yak and cattle which continues today in Asia (Shrode and Lush, 1947). Of the traits reported by Rege (1998), calving interval is perhaps most strongly correlated to thrift since during the stress of lactation the thrifty cow must be reproductively

active in order to initiate the succeeding lactation. This trait exhibited 9% heterosis in these data. Age at first calving determines when the cow first enters production, and the F1 exhibits 11% heterosis. On average, the F1 cow has her first calf only 2 weeks later than the best parental race. Superior thrift of the F_1 cow for milk production in the tropics for many situations cannot be questioned.

If recombination load is important for a trait the expected performance of groups having the same gene frequencies or racial makeup may vary considerably. A 5/8 Latin might be constructed by mating a Latin backcross to an F_1 or from a Greek backcross mated to a Latin purebred (Table 2). These differ considerably with respect to load. A further comparison of these with the 5/8 inter se suggests that the former cross would be unlikely to exhibit much decline upon inter se mating whereas the latter would. Syrstad (1989) discusses this case and gives examples from the literature.

Recombination load considered here differs somewhat from that usually considered in the population genetics literature wherein an equilibrium population is assumed with a desirable supergene sequence of, say, ABC. If in meiosis, some Abc and aBC gametes were produced, then zygotes resulting from such recombinant gametes would have less fitness causing a genetic load on the population. The recombination load considered here assumes a dynamic situation and a hybrid population with 'good,' coadapted sequences inherited from both parental taxa. The load occurs due to recombination destroying these sequences. Zebu and taurus do not rate full-species distinction and should be considered races in *Bos taurus* (Groves, 1981); however, they are sufficiently distantly related for reduced thrift to occur in the F_2. That zebu and taurus have long had separate ancestries has been recognized at least since Piggott's (1952) report of the two types being clearly distinguished in archeological material of the Indus Valley civilization (≈2600 BC). The extent of isolation was made much clearer by a recent examination of mitochondrial DNA sequence differences which indicate separate ancestries for 250 000 to 1 million years (Loftus et al., 1994). This is sufficient time to achieve diverse combinations of gene frequencies across a set of coadapted loci. It is not at present possible to document all the loci or the

alleles at those loci that contribute to genetic variability in milk production, but gene-mapping efforts and identification of major loci have begun in taurus (Barendse et al., 1994).

In Sewall Wright's view, evolution can proceed by partition of an evolving population into more or less isolated subpopulations in which chance plays a major role in determining genetic makeup and hence fitness of the subpopulations (Wright with Rutledge, 1978). Subpopulations of higher fitness flourish and eventually may supplant less-fit neighbors or they may evolve into separate species if barriers to crossing exist for sufficiently long periods. Wright believed that epistasis is a major contributor to fitness, and his shifting balance theory offers an easy way to envision the creation and fixation of favorable epistatic gene complexes. That is, by chance a particular population receives not only favorable alleles but perhaps as important favorable combinations of alleles from their founders which could subsequently become fixed.

The process of domestication practically insures that conditions for Wright's shifting balance theory will occur. In domestication, small isolates (captures from the wild) were interbred, probably at many locations and at many times (Diamond, 1997). The more successful isolates (those that could adapt to human husbandry and were productive) probably supplanted the less successful. Thousands of years later, the formal process of breed formation which again involved the inter- and in-breeding of small groups of founders again generated the necessary conditions for Wright's theory. In all likelihood, there were probably local landraces forming, crossing and reforming during the period between domestication and formal breed formation. Study of the formation of the Shorthorn breed and its early history was one of four key lines of evidence which led Wright to his view of evolution (Wright with Rutledge, 1978). It seems reasonable to assume that long-separated stocks of cattle, particularly domesticated cattle that have undergone accelerated evolution via artificial selection, may have merit due to epistasis that can be destroyed by recombination at meiosis.

Evidence developed herein along with decades of empirical experience suggests that creation of an adapted and productive milking breed based on a zebu×taurus crossbred is unlikely. Syrstad (1989) argued for a two-breed rotational cross using zebu and taurus sires in alternate generations. However, if epistasis is important in determining the crossbred means, performance may fall far below GTM prediction. The rotational cross does have the advantage of avoiding recombination loss of the third kind since one parent is always a purebred. At equilibrium for two loci about 45% of each generation are 'reconstituted F_1s', 10% are 'reconstituted purebreds' and the remainder incur a load of either the first or second kind depending upon the sire breed. McDowell (1994) champions temperate breeds and temperate technologies providing the nutritional, veterinary, climatic and husbandry needs of the cows can be satisfied. These are strong provisos and much suffering can result if the needs of exotic cows are not met (Ørskov, 1993). Breed replacement strategy may make political or economic sense, but often it is not ecologically sound. A recent and important review (Payne and Hodges, 1997) advocates use of F_1 sires which limits the proportion of taurus blood in the dairy cow population to one-half. If epistasis is important, then half the gametes of an F_1 sire carry an impaired sequence. This system is practiced widely in Latin America (Vaccaro et al., 1999) where most of the cows are mixed inter se animals with about equal representation from taurus and zebu. The physical and social, particularly economic, environments of the tropics exhibit great diversity; these in large part dictate the optimum strategy for any particular case. Undoubtedly there are circumstances where each of these strategies is optimal, and no single scheme should serve as a blanket policy.

In almost all cases, the F_1 has been superior (Cunningham and Syrstad, 1987). In the last decade, the technology of in vitro production of cattle embryos has matured from an experimental science to a technology (Gordon, 1994) that is ripe for exploitation. F_1 hybrids can be produced in almost unlimited quantities using oocytes from spent dairy cows (Rutledge, 1996) and semen from adapted breeds or even adapted species of cattle (McHugh and Rutledge, 1998). Biased sex ratios are possible using flow cytometry to sort sperm into X- and Y-bearing fractions (Cran et al., 1995) or other methods of sexing. A production system wherein a tropical dairyman initiates lactation in his F_1 cows

by transfer of an F_1 female in-vitro-produced embryo has no technological barriers. Such a system makes maximal use of heterosis and of complementarity and of the genetic resources in the genus Bos.

Acknowledgements

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