AN ASSOCIATION BETWEEN SERUM AMYLASE PHENOTYPE AND TICK INFESTATION IN CATTLE

By G. C. ASHTON,* G. W. SEIFERT,† and J. FRANCIS ‡

[Manuscript received September 6, 1967]

Summary

Twenty-five populations of cattle totalling 741 animals were analysed for serum amylase type. The number of ticks (*Boophilus microplus*) carried by these animals had been determined on various occasions prior to blood sampling. Tick burden was regressed on phenotype within populations and it was found that animals with the Am C phenotype carried significantly more ticks than other phenotypes.

I. INTRODUCTION

It has been shown by several groups of workers (Kelly 1943; Riek 1962; Francis and Little 1964) that in Queensland where the cattle tick *Boophilus microplus* is endemic, Zebu cattle are less infested than European breeds. While most workers agree that some part of this difference is genetic, heritability estimates obtained so far have not been consistent.

Although the chances of finding meaningful associations between specific phenotypes and quantitative characters appear small, Francis and Ashton (1967) nevertheless examined the relationship between tick count and phenotype in several known polymorphisms, viz. serum transferrins, serum post-albumins, serum amylases, haemoglobins, and J-antigens within one herd of Droughtmaster cattle. Surprisingly, the distribution of amylase alleles in the 20 least infested animals differed significantly from the distribution in the 20 most infested, the difference being due mainly to there being fewer Am^B alleles in the more infested group, or alternatively fewer Am^C alleles in the less infested.

This paper is concerned with the examination of a much larger number of cattle typed for serum amylase and counted for tick. The cattle were of diverse breeds and tick counts were estimated at different times in different locations. Nevertheless, an association between tick count and serum amylase phenotype was confirmed and extended.

* Department of Genetics, University of Hawaii, Honolulu, Hawaii 96822. Supported by U.S.P.H.S. Research Grant No. 1 RO1 HD 01831 of the National Institute for Child Health and Human Development.

† Division of Animal Genetics, CSIRO, "Belmont", Rockhampton, Qld. 4700.

[‡] Veterinary School, University of Queensland, St. Lucia, Qld. 4067.

II. MATERIALS AND METHODS

Experimental

Twenty-five populations of cattle were examined. Because of known variation in tick count due to sex, breed, season, location, etc., a population was considered as such only if all the factors known to affect tick count applied equally to each individual in the population. Cattle were located at "Amberley", Amberley, southern Queensland (populations 1–6 and 24–25), "Lansdown", Townsville, northern Queensland (populations 7–10), "Belmont", Rockhampton, central Queensland (populations 11–15 and 17–22) and Brisbane, southern Queensland (populations 16 and 23). Relevant data are given in Table 1.

Ticks were counted in the field by the method of Wilkinson (1955, 1962) in which adult female ticks 0.5 cm or more in length (excluding the capitulum) found on one side of the animal are included in the count. In some of the more recent work a modification of the technique developed by R. H. Wharton (unpublished) has been used. In this procedure only those ticks between 0.45 and 0.8 cm long are counted; the data for populations 17-22 were obtained in this manner.

Serum amylase phenotypes were determined by starch gel electrophoresis (Ashton 1965). There are six phenotypes Am A, Am B, Am C, Am AB, Am AC, and Am BC (referred to in the remainder of this paper as A, B, C, AB, AC, and BC). These represent respectively the three homozygous genotypes Am^{A}/Am^{A} , Am^{B}/Am^{B} , Am^{C}/Am^{C} , and the three heterozygous genotypes Am^{A}/Am^{C} , and Am^{B}/Am^{C} .

The serum typings and tick counts were done by different individuals, who had no knowledge of each other's results until the data were arranged for analysis.

Statistical Analysis

The data were analysed on a CDC 3100 computer in the Department of Genetics, University of Hawaii, using the multiple linear regression programme SUPEREG (Morton, Chung, and Mi, unpublished data 1967) kindly made available to us by Dr. M. P. Mi. This procedure is a leastsquares method and in the present application the analysis, carried out between populations, provides values for deviations of the remaining genotypes from one selected genotype. In the analysis presented here the genotype Am^C/Am^C is taken as baseline, and deviations of the tick count of the remaining five genotypes from that for Am^C/Am^C are presented. The analysis also provides the relevant standard errors of these estimates, and the significance of the genotype deviations are assessed by a conventional *t*-test.

A separate analysis was also made in which the mean value for the genotype Am^{C}/Am^{C} within populations was compared with the corresponding mean values for all other genotypes pooled.

III. Results

The numbers of, and mean tick counts for, each phenotype for each population are shown in Table 2. Considerable variation in mean tick count is apparent between populations. This is due to variation between breeds in susceptibility, differences in infestation levels at time of counting, and to variation in the number of counts represented in the total counts used in the data. There seems to be no generally acceptable way for weighting these variables, and the wide range in tick counts shown in Table 2 must be accepted as a feature of these data. Table 2 shows that in 54 out of a total of 73 comparisons Am^C/Am^C types carried more ticks on average than other genotypes.

Table 3 shows the results of the regression analyses. For the analysis involving all 25 populations C types carry the most ticks, and B, BC, AB, and AC types all carry significantly fewer ticks. Table 4 shows the means for C and non-C phenotypes.

The difference is significant, C carrying about 38% more ticks than the non-C types on the average.

		Amii	MOL III		
Popu- lation No.	Location	Breed	Sex	When Counted for Ticks, and Other Data	No.* of Animals
1	Amberley	A.I.S.†	Cows	Parents of populations 2–4	20
2	Amberley	A.I.S.	Cows	1962 progeny of population 1	12
3	Amberley	A.I.S.	Cows	1963 progeny of population 1	14
4	Amberley	A.I.S.	Cows	1964 progeny of population 1	11
5	Amberley	$\operatorname{Brahman} \times \operatorname{Hereford} F_1$	Steers	Born on Belmont, tested at Amberley	8
6	Amberley	$\mathbf{Africander} \times \mathbf{Hereford} \ \mathbf{F_1}$	Steers	Born on Belmont, tested at Amberley	8
7	Lansdown	Hereford	Cows	16 times between March and August 1965	19
8	Lansdown	Droughtmaster	Cows	19 times between March and July 1964. Breeders	38
9	Lansdown	Droughtmaster	Cows	Twice, November and December 1963. Classified cows	93
10	Lansdown	Droughtmaster	Cows	Twice, November and December 1963. Unclassified cows	136
11	Belmont	Brahman imes British‡ F ₂	Heifers	Three times, March 1963, Nov- ember 1963, and January 1964. Born November 1961	24
12	Belmont	$\operatorname{Brahman} \times \operatorname{British} F_2$	Steers	As population 12	23
13	Belmont	$A fricander imes British F_2$	Heifers	As population 12	26
14	Belmont	$A fricander imes British F_2$	Steers	As population 12	29
15	Belmont	$\mathbf{Shorthorn} \times \mathbf{Hereford} \mathbf{F}_2$	Heifers	As population 12	15
16	Brisbane	Droughtmaster	Cows	Numerous occasions in 1962. Population of Francis and Ashton (1967)	50
17	Belmont	$\operatorname{Africander} imes \operatorname{British} F_2$	Heifers	Seven times between Novem- ber 1965 and July 1966. Born November 1964	38
18	Belmont	$\operatorname{Brahman} imes \operatorname{British} \mathrm{F}_2$	Heifers	As population 17	19
19	Belmont	$\mathbf{Shorthorn} \times \mathbf{Hereford} \mathbf{F}_2$	Heifers	As population 17	23
20	Belmont		Steers	As population 17	57
21	Belmont	$\operatorname{Brahman} \times \operatorname{British} \mathrm{F_2}$	Steers	As population 17	26
22	Belmont	$\mathbf{Shorthorn} \times \mathbf{Hereford} \ \mathbf{F}_2$	Steers	As population 17	21
23	Brisbane	Friesian	Cows	As population 16	11
24	Amberley	Hereford	Cows		7
25	Amberley	Shorthorn	Cows		7

TABLE 1

details of the 25 populations of cattle counted for ticks and analysed for serum amylase type

* Total number 741.

† Australian Illawarra Shorthorn.

‡ British refers to a parental stock composed of crosses between Hereford and Shorthorn breeds.

Tables 3 and 4 also show the results of analyses in which the populations are grouped in different ways. If one considers populations in the same area then significant differences between genotype occur only in northern Queensland

TABLE 2

MEAN TICK COUNTS FOR EACH SERUM AMYLASE PHENOTYPE IN EACH POPULATION

See Table 1 for description of each population. Number of animals in each group given in parenthesis

Popu-	Mean Tick Count for Phenotypes:							
No.	A	AB	AC	В	BC	C	Count	
1	$5 \cdot 0 (1)$	$59 \cdot 0$ (1)	$44 \cdot 7$ (3)	69·0 (1)	89.6 (9)	63 .0 (6)	$69 \cdot 1$ (21)	
2		_			18.3(3)	40.8(9)	$35 \cdot 2$ (12)	
3			$9 \cdot 0$ (1)		$25 \cdot 6$ (5)	$35 \cdot 3$ (8)	30.0(14)	
4			20.0(2)		53.0(1)	18.1 (8)	$21 \cdot 6$ (11)	
5	$165 \cdot 0 (1)$	190.3(4)	$101 \cdot 0$ (1)	$379 \cdot 5 (2)$		_ `	$223 \cdot 3$ (8)	
6	60.0(1)	20.0(1)	67.5(2)	$109 \cdot 0$ (2)	399.5(2)		154.0 (8)	
7				$219 \cdot 8$ (5)	$403 \cdot 5(10)$	$438 \cdot 5$ (4)	362.5(19)	
8		$285 \cdot 0$ (2)		$188 \cdot 7(12)$	$173 \cdot 5(15)$	418.9(9)	$242 \cdot 3$ (38)	
9		$2 \cdot 0$ (3)	3.7(3)	$34 \cdot 7(31)$	$14 \cdot 1(43)$	$23 \cdot 5(13)$	21.5(93)	
10	$4 \cdot 0 (1)$	$5 \cdot 4$ (8)	6.5(4)	$17 \cdot 1(62)$	$9 \cdot 7(43)$	$23 \cdot 1(18)$	$14 \cdot 5(136)$	
11	17.0(5)	14.7(7)	14.6(7)		16.0(3)	29.0(2)	16.5(24)	
12	20.0(8)	$37 \cdot 3(7)$	36.5(4)	34.5(2)	16.0(2)		29.0(23)	
13	16.0(7)	18.0(1)	$18 \cdot 8(10)$		$25 \cdot 8$ (5)	$12 \cdot 0$ (3)	17.3 (26)	
14	$24 \cdot 3 (4)$	$34 \cdot 0 (2)$	$21 \cdot 4(12)$	49.0(1)	19.0(5)	$37 \cdot 4$ (5)	26.0(29)	
15			$7 \cdot 0$ (1)	37.0(1)	$46 \cdot 2$ (5)	$43 \cdot 1(13)$	41.7 (20)	
16	$252 \cdot 1$ (8)	$105 \cdot 7$ (7)	164.0 (8)	$59 \cdot 3$ (3)	$81 \cdot 2(14)$	$273 \cdot 2(10)$	$162 \cdot 3$ (50)	
17	$201 \cdot 3$ (3)		$346 \cdot 2(15)$	113.0(1)	$392 \cdot 1$ (9)	$243 \cdot 3(10)$	$312 \cdot 4$ (38)	
18	193.7(7)		179.7(6)		408.0(2)	$381 \cdot 8(4)$	$251 \cdot 4$ (19)	
19	270.0(1)	547.0(1)	_	400.0 (2)	$497 \cdot 9(16)$	584.7 (3)	$492 \cdot 9$ (23)	
20	172.5(4)	$75 \cdot 5 (4)$	$60 \cdot 4(24)$		$63 \cdot 5(12)$	$67 \cdot 5(13)$	71.6(57)	
21	$37 \cdot 2$ (8)	166.0(1)	$35 \cdot 8(8)$	•	$27 \cdot 6(5)$	46.0(4)	$41 \cdot 2$ (26)	
22			138.0(1)	$153 \cdot 7$ (3)	$111 \cdot 3 (9)$	$171 \cdot 1$ (8)	$141 \cdot 4$ (21)	
23		610.5(2)		519.0(4)	500.3(4)	$1881 \cdot 0$ (1)	$652 \cdot 7$ (11)	
24				$402 \cdot 0$ (1)	661.0(4)	$403 \cdot 5$ (2)	481.6 (7)	
25				_	$755 \cdot 0$ (1)	360.8 (6)	520.2 (7)	
Totals	(59)	(51)	— (112)	— (133)	(227)	— (159)	— (741)	

TABLE 3

AMYLASE TYPE AND TICK COUNTS FOR VARIOUS COMBINATIONS OF THE POPULATIONS SHOWN IN TABLES $1 \mbox{ and } 2$

Populations	No. of	No. of	Mean Count for C	Deviation of:					
ropulations	Animals	ations		$A ext{ from } C$	AB from C	AC from C	B from C	BC from C	
All	741	25	$154 \cdot 9$	-38.7	-49.5*	$-39 \cdot 4*$	$-55 \cdot 2^{**}$	-38.7**	
$\mathbf{Southern}$							1		
Queensland	129	10	$166 \cdot 8$	17.9	-86.9*	$-45 \cdot 2$	-58.9	-9.3	
Central									
Queensland	295	11	$135 \cdot 7$	$-24 \cdot 3$	$2 \cdot 0$	-11.0	$-40 \cdot 4$	$2 \cdot 6$	
Northern									
Queensland	282	4	$119 \cdot 9$	-61.0	$-53 \cdot 3$	$-63 \cdot 1$	$-57 \cdot 1***$	- 59 • 9***	
European	155	11	$282 \cdot 2$	-188.3	$-71 \cdot 2$	-44.0	$-156 \cdot 8**$	$-34 \cdot 3$	
Crossbred	561	14	$116 \cdot 3$	$-29 \cdot 9$	-44.5*	$-36 \cdot 2*$	-36.6*	-40.8**	
*P<0.05.		** $P < 0.01$.		*** $P < 0.001$.					

306

populations. In this area B and BC types carry significantly fewer ticks than C. The other types also carry fewer ticks but the differences are not significant. In the case of the southern and central Queensland populations none of the genotype differences are significant. However, 7 of the 10 deviations are negative and are consistent with the northern Queensland results. Table 4 shows similarly that C phenotypes carry significantly fewer ticks than non-C types in the northern Queensland populations. While the same effect is seen in the southern and central Queensland populations in neither case is the difference significant.

Populations	No. of Animals	Mean Count for Populations	$\begin{array}{c} \text{Count} \text{for} \\ \text{non-}C \ \text{Types} \end{array}$	$\begin{array}{c} \text{Count for} \\ C \text{ types} \end{array}$	t	Р
All	741	120.7	$111 \cdot 5 \pm 13 \cdot 3$	$154 \cdot 2 + 11 \cdot 6$	$3 \cdot 20$	< 0.01
Southern Queensland	129	$148 \cdot 0$	$140 \cdot 0 \pm 33 \cdot 4$	$162 \cdot 6 \pm 25 \cdot 6$	0.68	n.s.
Central Queensland	295	$128 \cdot 2$	$126 \cdot 4 \pm 19 \cdot 5$	$135 \cdot 2 \pm 17 \cdot 1$	0.45	n.s.
Northern Queensland	282	70.5	$61 \cdot 5 \pm 15 \cdot 7$	$119 \cdot 9 + 14 \cdot 5$	$3 \cdot 72$	<0.001
European	155	$246 \cdot 2$	$223 \cdot 9 \pm 35 \cdot 1$	$278 \cdot 4 \pm 26 \cdot 0$	1.55	n.s.
Crossbred	561	84.4	$78 \cdot 4 \pm 13 \cdot 4$	$116 \cdot 2 \pm 12 \cdot 3$	$2 \cdot 82$	< 0.01

Table 4 mean tick counts and standard errors for C and non-C types

If the populations are grouped on the basis of breed into *Bos taurus* and *B. taurus* $\times B$. *indicus* cattle the results are similar to the area grouping. The crossbred cattle have AB, AC, B, and BC types carrying significantly fewer ticks than C (Table 3), and C carrying fewer ticks than non-C types (Table 4). In the case of the *B. taurus* cattle only, B types carry significantly fewer ticks than C cattle. However, the deviations are negative for all the other types though the effects are not significant.

IV. DISCUSSION

The results reveal a significant difference overall in the tick burden carried by C cattle compared with non-C cattle. Preliminary results (Francis and Ashton 1967) had prompted the suggestion that animals with the allele Am^B may carry fewer ticks than animals lacking it. The more extensive data presented here support this conclusion. Thus B, BC, and AB phenotypes, carrying the allele Am^B , have significantly fewer ticks in the main comparison (Table 3) than C which lacks it. However, the more correct interpretation may be that C phenotypes carry more ticks on average than the other five phenotypes, because A and AC also carry fewer ticks than C, but do not possess Am^B .

The results seem consistent between areas but significant differences are seen only in the northern Queensland cattle. In this area 282 animals from four populations show highly significant differences between the infestation carried by C cattle and B and BC cattle. In the breed analysis the greater burden of C is significant in the crossbreeds, but not in the British cattle, although the trend is consistent. Nearly half of the total of 561 crossbred cattle come from populations 8, 9, and 10, i.e. the northern Queensland herds. Although the results appear consistent it is clear that these three populations contribute greatly to the significant differences found in the analysis of the data. Numerically populations 9 and 10 are the largest and account for one-third of the cattle in the analysis. This may be the reason for their preponderant effect. Fortuitous associations between genotype and quantitative characters are to be expected, and are only countered by repetition of the association in diverse populations. Table 2 provides evidence that the C-non-C difference is probably not fortuitous.

The extent of the difference in tick burden is seen in Table 4. The C animals carry from about 7% more ticks than the non-C animals (central Queensland population) up to about twice as many ticks (northern Queensland population).

The nature of the effect of course is unknown, and one can only speculate on the mechanism whereby serum amylase genotype affects infestation of an animal by tick. From the genetic viewpoint it seems likely that C animals will be subject to a fitness disadvantage in a climate where tick is endemic. It is known that gain in body weight decreases with increasing tick burden (Norman 1960; Little 1963). Seifert and Riek (unpublished data), running Zebu crossbred and British cattle together, have shown a severe effect of tick on the body weight of the British animals. Two out of five British castrate males were lost due to the direct effect of tick, while body weight losses of 80–100 lb were experienced over a 6-week period. Body-weight losses for Zebu crossbreds were insignificant. There are no data relating tick burden to fertility. If, as seems likely, heavily infested cows proved to be less fertile than those lightly infested, some mechanism would be necessary to retain Am^C in a breed inhabiting an endemic area.

V. Acknowledgments

Thanks are due to Dr. H. Wharton, Veterinary Parasitology Laboratory, CSIRO, Yeerongpilly, Qld., and to Mr. J. B. Ritson and Mr. L. A. Y. Johnston, Division of Tropical Pastures, CSIRO, Townsville, Qld., for blood samples from cattle at Amberley and Landsown, and for their generous provision of tick-count data on these animals. Mr. M. N. Dennis gave capable technical assistance.

VI. References

ASHTON, G. C. (1965).—Genetics, Princeton 51, 431-7.

FRANCIS, J., and ASHTON, G. C. (1967).—Aust. J. exp. Biol. med. Sci. 45, 131-40.

FRANCIS, J., and LITTLE, D. A. (1964).-Aust. vet. J. 40, 247-53.

- KELLY, R. B. (1943).—Bull. Coun. Sci. Industr. Res. Aust. No. 172.
- LITTLE, D. A. (1963).—Aust. vet. J. 39, 6–10.
- NORMAN, M. J. T. (1960).-CSIRO Aust. Div. Land Res. Reg. Surv. Tech. Pap. No. 12.
- RIEK, R. F. (1962).—Aust. J. agric. Res. 13, 532-50.

WILKINSON, P. R. (1955).—Aust. J. agric. Res. 6, 655-65.

WILKINSON, P. R. (1962).-Aust. J. agric. Res. 13, 974-83.