TABLE 3 Comparisons of events occurring during rejections ($n = 26$) and acceptances

	Acceptance*	Rejection	P
Male or female aggression	1/17	3/26	>0.10†
Female ran from/ evaded male	11/17	17/26	>0.10‡
Female did not evade male:			
Male attempts mount	6/6	0/9	
Male leaves	0/6	8/9	
Total male approaches	1.88 (s.d. = 1.27)	0.80 (s.d. = 0.91)	<0.01§
Male approach rate (per min)	0.50 (s.d. = 0.41)	0.21 (s.d. = 0.29)	<0.01‖

* The acceptance cases considered here were those in which no competitor was present ($n = 17$); male-female interactions before the male's first mount, which occurred an average of 8.9 (s.d. = 12.91) min after his arrival, are compared with the interactions occurring before rejecting males departed from the female.
† $X^2 = 0.008$, 1 d.f.
‡ $X^2 = 0.079$, 1 d.f.
§ Mann-Whitney $U = 100$, $z = 3.00$.
‖ Mann-Whitney $U = 114$, $z = 2.66$.

that mated with at least three males, to determine whether females became less receptive[20] with either increases in delay and/or sexual activity. Although third males began mating at an average delay of 3.2 h (s.d. = 1.09), females terminated roughly the same percentage of copulations with them as they had with first males ($\bar{x} = 81.1\%$ for first male copulations; 80.6% for third mates; analyses include only copulations unambiguously terminated by either participant; Mann-Whitney $U = 53.5$; $P > 0.10$). Second, we compared female behaviour during rejections and acceptances (Table 3), and found no differences in aggressiveness or evasiveness. Instead, rejections were clearly distinguishable by a lack of male persistence (Table 3). In contrast to the mean of 2.1 h that males spent pursuing females with which they mated, male visits with rejected females lasted only 6.0 min (s.e. = 0.98). Rejected females often (15/26 cases) either reinitiated contact with a male by approaching him, or attempted to prolong contact by following him as he departed. Overall, our impression is that rejected females were still willing to mate, but were no longer sexually attractive[20].

According to recent arguments, the advantage to males of mating selectivity depends on two key features: variation in female quality and costs of obtaining alternative mates[2,4,6]. As applied here to thirteen-lined ground squirrels, male choice should be favoured, even in the absence of paternal investment and despite high search costs, because of predictable variation in female quality arising from sperm competition. It seems possible that male choosiness, as mediated by cues regulating male sexual interest, may commonly be driven by sperm competition and, in turn, may frequently limit the duration of behavioural oestrus in female mammals. A lack of male attraction to females at particular stages of oestrus (as revealed by either male-initiated termination of pursuit or male refusal to mate) has been noted in field studies of several other mammalian species[21-23]. □

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Molecular and phenotypic variation in the *achaete-scute* region of *Drosophila melanogaster*

Trudy F. C. Mackay* & C. H. Langley†

* Department of Genetics, Box 7614, North Carolina State University, Raleigh, North Carolina 27695-7614, USA
† Center for Population Biology and Department of Genetics, University of California, Davis, California 95616, USA

VARIATION in quantitative characters underlies much adaptive evolution and provides the basis for selective improvement of domestic species, yet the genetic nature of quantitative variation is poorly understood[1]. Many loci affecting quantitative traits have been identified by the segregation of mutant alleles with major qualitative effects[2,3]. These alleles may represent an extreme of a continuum of allelic effects, and most quantitative variation could result from the segregation of alleles with subtle effects at loci identified by alleles with major effects[4-6]. The *achaete-scute* complex in *Drosophila melanogaster* plays a central part in bristle development[7,8] and has been characterized at the molecular level[9,10]. The hypothesis that naturally occurring quantitative variation in bristle number could be associated with wild-type alleles of *achaete-scute* was tested by correlating phenotypic variation in bristle number with molecular variation in restriction maps in this region among chromosomes extracted from natural populations. DNA insertion variation in the *achaete-scute* region was found to be strongly associated with variation in bristle number.

The restriction map variation of a 106-kilobase region including the *achaete-scute* complex has been quantified among 64 isogenic X-chromosome lines extracted from three natural *D. melanogaster* populations[11]. Thirty-six of the X chromosomes from two of the populations (Raleigh and Texas) studied in this survey were still available (from C. C. Laurie, Duke University). Variation among these chromosomes for abdominal and sternopleural bristle number was assessed. The autosomes of all X chromosome lines were isogenic (see ref. 12 for details), so variation in bristle score among the lines is due to variation at X chromosome loci. There are 17 restriction map polymorphisms in the *achaete-scute* region among the 36 lines, with 4 polymorphic restriction sites, 11 insertions and 2 deletions. Of these polymorphisms, only 6 have frequencies greater than 0.10. Because of such low heterozygosities, and the large amount of phenotypic variation for the bristle traits, we did not attempt to associate presence or absence of individual molecular variants with variation in bristle score. As known bristle mutants are associated with lesions in the *achaete-scute* region[9,13], we tested whether the insertions in the naturally occurring *achaete-scute* alleles were associated with phenotypic variation in abdominal and sternopleural bristle number. Of the 13 haplotypes represented in the sample, 9 have at least one insertion. The insertion haplotypes are represented by 16 of 36 lines.

The mean rank order bristle scores of the 36 X-chromosome lines and their insertion haplotypes are shown in Fig. 1. Averaged over both populations, lines with insertions have 1.62 fewer sternopleural bristles and 1.18 fewer abdominal bristles than

lines without insertions. This difference was highly significant when tested by analysis of variance (Table 1). In terms of additive genetic (σ_a) and phenotypic (σ_p) standard deviation units, the reduction in bristle number for haplotypes containing insertions compared with insertion-free haplotypes is $1.62\sigma_a$ and $0.73\sigma_p$ for sternopleural bristles, and $1.53\sigma_a$ and $0.58\sigma_p$ for abdominal bristles.

The significant difference in overall female and male score of 0.74 sternopleural and 2.89 abdominal bristles is typical of sexual dimorphism for this species. The sex dimorphism in bristle number varies significantly among lines for both traits, suggesting that genes on the X chromosome affecting bristle number have differential effects on the two sexes. Interestingly, the presence or absence of insertions in *achaete-scute* has a significant effect on the sex dimorphism of abdominal bristle number (see Table 1; sex by insertion interaction).

To our knowledge, this is the first evidence that insertions in natural populations are associated with quantitative morphological variation, although there is evidence that new *P*-element insertions cause variation for bristle number and other quantitative traits[14-16]. The fraction of the X-chromosomal variation for bristle number attributable to insertional variation at *achaete-scute* is surprisingly large. If we assume all insertion haplotypes have similar additive effects in reducing bristle score, and the insertion haplotypes are in Hardy-Weinberg equilibrium, then the variance contributed by insertions at *achaete-scute* in a random-mating population is $a^2q(1-q)/2$, where a is the difference in mean bristle number between homozygous insertion and non-insertion haplotypes, and q is the frequency of insertion haplotypes. The additive variance due to insertions at *achaete-scute* is thus 0.323 for sternopleural and 0.172 for abdominal bristle number, respectively; and the proportion of

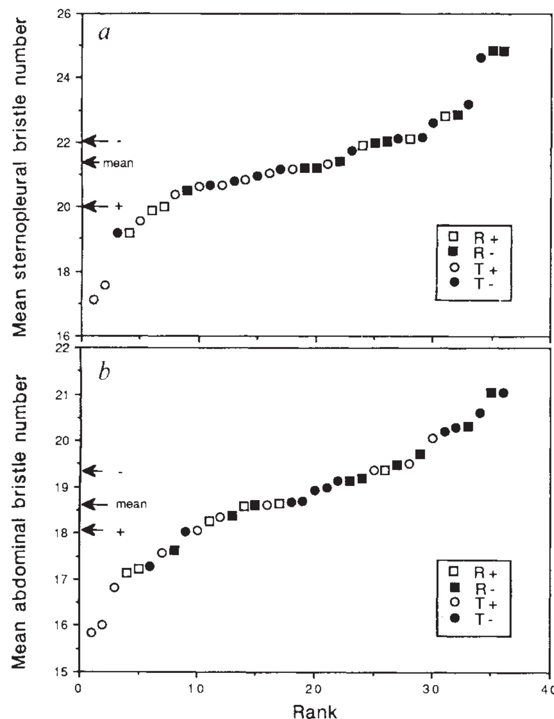

FIG. 1 Rank order of mean sternopleural (*a*) and abdominal (*b*) bristle scores from isogenic X chromosome lines extracted from the Texas (T) and Raleigh (R) natural populations[12]. The total number of sternopleural bristles on the right and left sternopleural plates, and the number of abdominal bristles on the most posterior abdominal sternite, was recorded on a sample of 10 males and 10 females from each of 2 replicate vials per line. Each line mean is the average of these 40 measurements. The presence of an insertion in the *yellow-achaete-scute* region[11] is indicated in the box by +, and no insertion in the region by −. The arrows on the ordinate of the plots indicate the mean bristle scores of all lines (mean), of lines without insertions (−) and of lines with insertions (+).

TABLE 1 Analysis of variance of sternopleural and abdominal bristle number

Source of variation	d.f.	Sternopleural bristles Mean square	Sternopleural bristles F	Abdominal bristles Mean square	Abdominal bristles F
Sex	1	195.81	24.07*	3,013.12	351.18*
Pop	1	264.19	2.92^{ns}	11.13	0.20^{ns}
Ins	1	856.53	9.46†	484.35	8.66†
Pop × Ins	1	13.82	0.15^{ns}	2.22	0.04^{ns}
Sex × Pop	1	0.39	0.05^{ns}	0.10	0.01^{ns}
Sex × Ins	1	0.10	0.01^{ns}	62.99	7.34‡
Sex × Pop × Ins	1	0.14	0.02^{ns}	3.01	0.35^{ns}
Line (Pop × Ins)	32	90.51	13.76*	55.91	12.14*
Sex × Line (Pop × Ins)	32	8.14	1.81‡	8.58	1.74‡
Rep (Line)	36	6.58	1.46^{ns}	4.61	0.93^{ns}
Sex × Rep (Line)	36	4.50	1.30^{ns}	4.94	1.54‡
Error	1,296	3.47		3.22	

Variance was partitioned into sources attributable to main independent fixed effects due to sex (Sex), population (Pop), insertion categories (Ins) and their two- and three-way interactions; the random effect of chromosome line (Line) nested within population by insertion category; the random effect of replicate vial (Rep) nested within chromosome line; sex by line within population by insertion class and sex by replicate within line interactions; and error. The analysis was performed using SAS variance components estimation procedure. The variance component among homozygous X-chromosome lines (within population and insertion categories) estimates twice the X-chromosomal genetic variance for bristle score of a non-inbred population, assuming complete additivity. The estimate of additive genetic variance due to loci on the X chromosome from this analysis is thus 1.004 for sternopleural and 0.596 for abdominal bristle number. The phenotypic variance is estimated from the sum of all variance components, and is 4.859 for sternopleural and 4.168 for abdominal bristle number. The square root of the additive genetic (phenotypic) variance gives the additive genetic (phenotypic) standard deviation units used in the text to scale the effect of insertion haplotypes on bristle score.

* $P<0.001$; † $0.001<P<0.01$; ‡ $0.01<P<0.05$; ns, $P>0.05$.

the total X-chromosome genetic variance due to effects of inserts at *achaete-scute* is 0.243 for sternopleural bristles and 0.224 for abdominal bristles. As the X chromosome is roughly one-fifth of the genome, about 5% of the total genetic variance of abdominal and sternopleural bristle number might be caused by the effects of insertions at this locus.

This analysis assumes that *achaete-scute* is in linkage equilibrium with other X-chromosome loci. Because *achaete-scute* is located at the tip of the X chromosome, in an area of reduced recombination[11], it is possible that the observed effect is caused by linkage disequilibrium between *achaete-scute* and linked loci. But the restriction of recombination does not extend beyond the *zeste-white* region, for which pairs of closely linked polymorphic restriction sites show little linkage disequilibrium[11], so any linkage disequilibrium involves only loci in a small region of the X chromosome immediately adjacent to *achaete-scute*.

We have shown that naturally occurring molecular variation at a major bristle locus, the *achaete-scute* complex, can be associated with nearly 5% of the total quantitative genetic variation for bristle number. It is likely that a small number of loci contribute most of the genetic variation for any quantiative trait, with more numerous loci contributing less[17,18]. This analysis suggests candidates for the loci with most effect on a trait may be loci identified by major qualitative mutations affecting fundamental developmental processes, and that the approach taken here with *achaete-scute* may be applicable in general to the study of quantitative genetic variation. The nature of the restriction map variation associated with the phenotypic variation is intriguing. It is important to determine whether naturally occurring insertions generally have detectable phenotypic effects, and if so, if they commonly reduce trait values. The *achaete-scute* complex may be peculiar because of its large regulatory regions[13], its interactions with genes involved in sex determination[19], and because of its location at the tip of the X chromosome[11]. □

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Stimulation of the phosphatidylinositol pathway can induce T-cell activation

Dev M. Desai, Marianne E. Newton, Theresa Kadlecek & Arthur Weiss

Howard Hughes Medical Institute, Departments of Medicine and of Microbiology and Immunology, University of California at San Francisco, San Francisco, California 94143, USA

THE T-cell antigen receptor (TCR) regulates two signal transduction pathways: the phosphatidylinositol (PtdIns)[1] and tyrosine kinase pathways[2]. Stimulation of T cells with antigen or anti-TCR monoclonal antibodies induces an increase in inositol phosphates and diacylglycerol[1,3], the second messengers responsible for the mobilization of cytoplasmic free calcium and activation of protein kinase C[4]. The TCR also activates a tyrosine kinase that is not intrinsic to the TCR[2]. The relationship between these two signal transduction pathways and their contribution to later T-cell responses is unclear. Studies using variants of a murine hybridoma suggested that the PtdIns pathway might not be necessary for or be involved in regulating interleukin-2 (IL-2) production[5]. To address the relationship between later T-cell responses and the early biochemical signals, we investigated the ability of a heterologous receptor with defined signal transduction function to induce T-cell activation. The human muscarinic subtype-1 receptor (HM1)[6], which elicits PtdIns metabolism in neuronal cells through a G protein-coupled mechanism[7], also functionally activates this pathway when expressed in the T-cell line Jurkat-derived host, J-HM1-2.2 (ref. 8). We show here that stimulation of HM1 alone induced IL-2 production and IL-2 receptor α chain expression. HM1 does not induce the tyrosine kinase pathway, suggesting that this pathway does not directly influence later T cell-activation responses. Instead, our studies indicate that activation of the PtdIns pathway is probably sufficient to induce later T-cell responses.

Comparison of the time-dependent appearance of inositol phosphates revealed that Jurkat T cells responded well to stimulation by C305, an agonist anti-TCR monoclonal antibody, but were unresponsive to the muscarinic receptor agonist carbachol (Fig. 1*a*), consistent with its failure to express muscarinic binding sites[8]. J-HM1-2.2, which expresses both receptors, increased all three inositol phosphate fractions in response to either C305 or carbachol (Fig. 1*b*). When HM1 was stimulated, however, the total increase of inositol phosphates was substantially greater and their temporal accumulation was more prolonged than in the TCR-induced response.

As comparable numbers of TCR and HM1 are expressed on J-HM1-2.2 (ref. 8), the differences in observed responses must be attributable to other differences between these receptors. The TCR is known to be internalized following exposure to soluble anti-TCR monoclonal antibody[9,10]. Unlike the TCR, whose expression decreases substantially during stimulation, expression of HM1 is not diminished by carbachol stimulation (Fig. 1*b*). Hence, failure of HM1 to internalize may contribute to the larger and more sustained increases in inositol phosphates following ligand binding. In contrast, the more transient inositol phosphate increases induced by the TCR are likely to be attributable, in part, to its rapid internalization after binding soluble

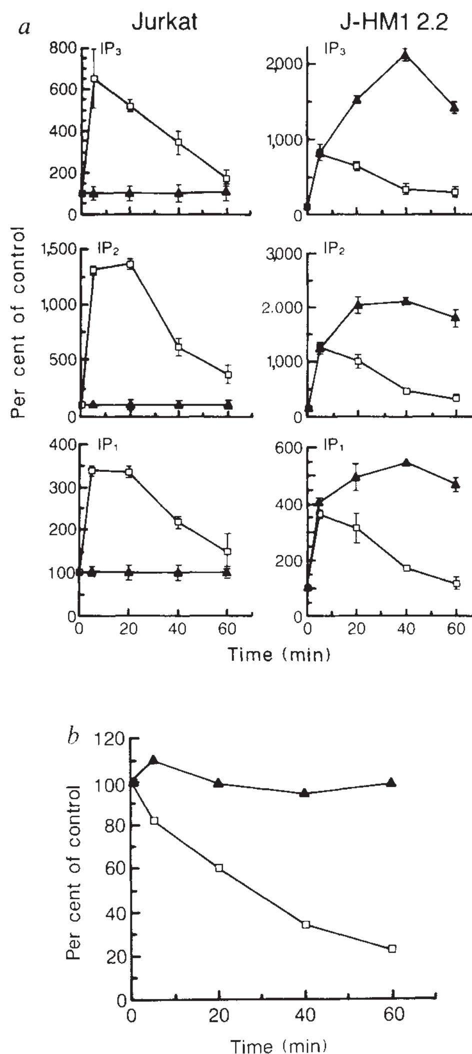

FIG. 1 *a*, Jurkat or J-HM1-2.2 cells were stimulated with anti-TCR monoclonal antibody (mAb)C305 (□) or carbachol (▲) and the indicated inositol fractions were assayed at the times indicated. IP_3, inositol-1,4,5-trisphosphate; IP_2, inositol-1,4-bisphosphate; IP_1, inositol-1-monophosphate. *b*, J-HM1-2.2 cells were stimulated with saturating amounts of either C305 (□) or carbachol (▲) as in *a* in medium at 37 °C. At various times, cells were removed and the relative levels of the TCR or HM1 receptors remaining on the cell surface were determined.

METHODS. *a*, Inositol phosphate levels in Jurkat and J-HM1-2.2 following stimulation were determined as described[8]. Cells were stimulated with either C305 (1/1,000 dilution of ascitic fluid, a saturating concentration) or carbachol (500 μM, a saturating concentration). Results are expressed as the mean % of control (unstimulated cells) ± s.e.m. *b*, Relative levels of the TCR or HM1 receptors remaining on the cell surface after incubation with anti-TCR (C305) or carbachol were determined by immunofluorescence with fluorescein-conjugated anti-Leu 4 mAb (anti-CD3) and flow cytometry or by saturable binding studies with the muscarinic antagonist [^{3}H]quinuclidyl benzilate (QNB), respectively[8]. Results are expressed as the mean % of the level of relevant receptor expression on unstimulated cells from three separate experiments.