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12. Nucleotide differences between 16S-like rRNAs of the attine fungal symbionts are consistent with a phylogenetic analyses based on partial 28S-like rRNA sequence determinations from genera corresponding to those included in this study [I. H. Chapela, S. A. Rehner, T. R. Schultz, U. G. Mueller, *Science* **266**, 1691 (1994)].
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17. Maximum parsimony analysis (with branch and bound search strategies) produced two trees of 426 steps. Both trees contain the five attine fungal symbionts analyzed in a coherent phylogenetic assemblage. However, bootstrap parsimony (*14*), maximum likelihood (*15*), and distance matrix (*16*) methods do not identify a preferred branching order among *Agaricus*, *Lepiota*, and the fungal symbiont of *Apterostigma*.
18. If it had been possible to demonstrate that the fungal symbiont of *Apterostigma* branched before the divergence of *Lepiota* and *Agaricus*, two competing interpretations would have had to have been considered. In the first, two independent origins of symbiotic fungi with attine ants may have occurred. In the alternative interpretation, *Lepiota* and *Agaricus* may have escaped the symbioses in favor of a free-living life-style.
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13 June 1994; accepted 21 October 1994

Naturally Occurring Variation in Bristle Number and DNA Polymorphisms at the *scabrous* Locus of *Drosophila melanogaster*

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The association between quantitative genetic variation in bristle number and molecular variation at a candidate neurogenic locus, *scabrous*, was examined in *Drosophila melanogaster*. Approximately 32 percent of the genetic variation in abdominal bristle number (21 percent for sternopleural bristle number) among 47 second chromosomes from a natural population was correlated with DNA sequence polymorphisms at this locus. Several polymorphic sites associated with large phenotypic effects occurred at intermediate frequency. Quantitative genetic variation in natural populations caused by alleles that have large effects at a few loci and that segregate at intermediate frequencies conflicts with the classical infinitesimal model of the genetic basis of quantitative variation.

Knowledge of the genetic basis of quantitative characters is important with regard to medicine, the improvement of domestic species, and our understanding of evolution, yet little is known about the particular Mendelian variants that give rise to the heritable component of these traits. One hypothesis is that allelic variation at loci important in the development of a particular trait is a major source of quantitative differences in that trait (*1*). The numbers of abdominal and sternopleural bristles of *Drosophila melanogaster* are typical quantitative characters (*2*). Because *Drosophila* bristles are sensilla (sensory organs) of the peripheral nervous system, candidate genes for bristle number traits are the 10 to 20 proneural and neurogenic loci that determine the presence or absence of sensory hairs (*3*). Alleles of large effect at some of these loci may contribute to the response to artificial selection for high and low bristle numbers (*4*), and insertional polymorphisms in the proneural *achaete-scute* complex (ASC) are associated with naturally occurring genetic variation in bristle number (*1*). The *scabrous* (*sca*) locus encodes a signal protein important in lateral inhibition of the developing nervous system, and mutant *sca* alleles have large effects on bristle number and eye morphology (*5*). We have now tested the hypothesis that allelic variation at *sca* contributes to quantitative genetic variation in natural populations of *D. melanogaster* by associating molecular polymorphisms at this locus with genetic variation in bristle number.

We determined the mean abdominal and sternopleural bristle numbers for each sex from 47 independent second chromosome lines extracted from a natural population and placed in an isogenic genetic background (*6*) (Table 1). The overall means (±SE) were 16.30 ± 0.42 abdominal bristles and 16.15 ± 0.22 sternopleural bristles. Assuming additivity, the total additive genetic variance (σ^2_A) of the second chromosome was estimated as 1.23 for abdominal bristle number and 0.64 for sternopleural bristle number (*7*). The additive genetic covariance between both characters was 0.39, with a genetic correlation coefficient of 0.43 (*7*). These estimates are consistent with previous observations (*8*).

Restriction map variation of a 45-kb region including the *sca* locus was quantified among the 47 chromosomes (Fig. 1 and Table 1). There were 18 restriction site polymorphisms and 25 length polymorphisms (insertions and deletions). Single-stranded conformation polymorphism (SSCP) was determined (*9*) for three fragments that encompass the last intron and

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parts of the third and fourth exons (Fig. 1); 31 classes of SSCP were observed. The estimated nucleotide diversity (π) for the entire 45-kb region was 0.010 (*10*). Some small insertions and deletions (<0.5 kb) were present at intermediate frequencies, whereas large insertions (>0.5 kb) were rare, which is consistent with previous surveys (*11*). Levels of pairwise linkage disequilibria among the 27 polymorphic sites with frequencies between 3/47 and 44/47 were analyzed (Fig. 1). This amount and pattern of molecular variation are typical for *D. melanogaster* loci (*11*).

We tested whether the 27 polymorphic sites with frequencies between 3/47 and 44/47 (*12*) and the presence or absence of large insertions (>0.5 kb) (Table 1) were associated with bristle number variation using a linear model (*13*). Eleven sites showed a significant ($P < 0.05$) or a highly significant ($P < 0.01$) association with variation in one of the two bristle traits for their average effect (the main effect) or sex dimorphic effect (the sex*site interaction) (Table 2). The probability of observing 11 significant sites out of 112 tests was assessed by random permutation of the molecular haplotypes among phenotypes. In 1000 such permutations, 957 had ten or fewer significant associations of polymorphic sites with bristle number for either the main effect or the sex*site interaction, which indicates that some molecular polymorphisms in the *sca* region are significantly associated with variation in abdominal or sternopleural bristle number (or both) (*14*). The main effects of polymorphic sites DelE(−2.6), Eco RI(6.5), Pst I(26.6), SSCP 1447, and SSCP 1683, as well as the sex*site interactions of InF(−2.0), were significantly ($P < 0.05$) correlated with variation in abdominal bristle number, whereas the main effects of Pst I(−12.0), Eco RI(−1.5), and InL(5.3), and the sex*site interactions of Eco RI(12.0)−1.8 and Eco RI(14.5), showed significant associations with variation in sternopleural bristle number. The mean bristle number of the lines with large insertions (>0.5 kb) in the *sca* region was not significantly different from that of the lines without large insertions ($P > 0.93$ and $P > 0.77$ for sternopleural and abdominal bristle numbers, respectively). The sites associated with variation in abdominal bristle number were different from those associated with variation in sterno-

Table 1. Mean bristle numbers of the homozygous second chromosomes lines and their molecular variation in the *scabrous* region. For diallelic polymorphic sites (restriction enzyme polymorphisms and deletions or insertions): + present; − absent. Rare polymorphic sites listed in the fourth column from the right are: 1, InA(−14.1); 6, Pst I(−5.2); 10, Pst I(−1.6); 12, InG(1.2); 13, InH(1.5); 14, InI(3.1); 16, InK(4.7); 17, Bam HI(4.7); 22, Eco RI(11.5)−1.3; 26, DelN(13.5); 27, InO(14.7); 30, InQ(20.0); 31, InR(21.2); 33, InT(20.2); 35, InU(25.3); 36, InV(24.7); 39, Pst I(31.0); and 42, InX(30.6). The AB(1) haplotype is the abdominal bristle number haplotype based on Eco RI(6.5), Pst I(26.6), and SSCP 1447; the AB(2) haplotype is the abdominal bristle number haplotype based on Eco RI(6.5) and Pst I(26.6) only; and the SB haplotype is the sternopleural bristle number haplotype based on Pst I(−12.0), Eco RI(−1.5), and InL(5.3). Unique haplotypes were pooled into one haplotype class. Diallelic polymorphism frequencies are shown in the last row. The nine SSCP 1447 alleles were grouped into six classes: 1 (22), 2 (5), 3 (8), 4 (6), 8 (2), and 5 (4), where the number in parentheses is the number of chromosomes of each allelic class and the last class listed is composed of all unique alleles. Likewise, the eight SSCP 1683 alleles were grouped into six classes [1 (26), 2 (6), 3 (7), 4 (3), 6 (2), 5 (3)] and the 14 SSCP 1836 alleles into eight classes [0 (4), 1 (23), 3 (3), 4 (2), 5 (3), 6 (3), 10 (2), and 2 (7)].

Chromosome line	Abdominal bristle		Sternopleural bristle		DelB(−10.8)	Pst I(−12.0)	InC(−5.9)	DelD(−5.4)	Pst I(−3.9)	DelE(−2.6)	InF(−2.0)	Eco RI(−1.5)	DelJ(3.8)	InL(5.3)	Pst I(5.8)	Eco RI(8.5)	DelM(7.8)	Eco RI(11.5)-1.5	Eco RI(11.9)-1.7	Eco RI(12.0)-1.8	Eco RI(14.5)	DelS(21.7)	Eco RI(20.2)	Pst I(26.6)	DelW(27.6)	Bam HI(27.0)	Bam HI(31.5)-3.8	InY(32.1)	SSCP1447	SSCP1683	SSCP1836	Insertion >0.5 kb	Rare (N < 3) polymorphism	AB(1) haplotype	AB(2) haplotype	SB haplotype
	Male	Female	Male	Female																															
1	17.00	18.10	16.15	17.90	-	-	-	+	+	-	-	+	-	-	+	+	+	+	-	-	+	-	-	-	+	+	-	1	1	1	-		1	1	1
2	15.25	16.30	17.00	17.75	-	-	-	-	+	-	-	+	-	-	-	+	-	+	-	-	+	-	-	-	+	-	-	1	1	1	-		1	1	1
5	16.80	17.60	18.95	20.35	-	-	-	-	+	-	-	+	-	-	-	+	-	+	-	-	+	+	-	-	+	+	+	1	1	7	-		1	1	1
7	16.75	18.85	16.00	16.95	-	-	-	-	+	-	-	+	-	-	+	+	-	-	+	-	+	-	-	-	+	+	+	3	2	5	-		2	1	1
9	15.35	17.30	15.75	16.25	-	-	-	+	+	-	-	+	-	-	-	+	-	+	-	-	-	-	-	+	-	-	-	1	1	1	-	39	3	2	1
10	13.75	15.70	15.35	15.40	-	+	-	-	-	-	-	-	-	-	-	+	-	+	-	-	+	-	-	+	-	-	-	1	1	1	-		3	2	5
12	16.30	17.45	14.75	18.75	+	-	-	-	+	-	-	+	-	-	+	+	-	-	+	-	+	-	+	-	-	-	+	3	2	8	-		2	1	1
15	13.60	17.55	14.95	16.15	-	+	+	-	+	-	+	+	-	-	-	+	-	+	-	-	+	-	-	-	-	+	-	1	1	5	+	14,30	1	1	2
16	16.10	18.30	14.40	16.15	-	-	-	-	+	-	-	+	-	-	-	+	-	-	-	-	+	-	-	-	-	-	+	4	1	3	-		5	1	1
17	15.40	15.80	14.70	15.00	-	+	-	-	+	-	-	+	-	+	+	+	-	-	-	-	+	-	-	-	+	-	-	1	1	1	-	22	1	1	6
18	14.40	17.25	13.30	15.10	+	+	-	-	-	-	-	-	-	-	-	+	-	+	-	-	+	-	-	+	-	+	-	1	1	1	-		3	2	5
19	13.85	18.55	13.50	15.00	-	-	-	+	-	-	-	-	-	-	-	+	-	+	-	-	+	-	-	+	-	-	-	1	1	5	-		3	2	4
22	13.30	15.70	16.00	16.90	-	-	-	-	-	-	-	+	-	-	+	-	-	-	-	+	-	-	-	-	+	-	-	4	3	3	-		7	4	1
25	16.90	17.80	17.35	18.05	+	-	-	-	-	-	-	+	-	-	-	+	-	-	-	+	-	-	-	+	-	+	-	3	2	9	-		7	2	1
30	17.15	19.55	14.50	15.65	+	-	-	-	-	-	-	+	-	+	-	+	-	+	-	-	-	-	-	-	-	+	+	8	1	1	-		7	1	3
32	20.25	21.15	16.75	18.60	-	-	-	-	+	-	-	+	-	-	-	+	+	+	-	-	+	-	-	-	+	-	-	2	1	1	-		8	1	1
35	15.35	17.40	15.85	17.75	+	-	-	-	+	-	-	+	-	-	-	+	-	+	-	-	+	-	-	-	-	-	+	1	1	1	+	12	1	1	1
36	15.10	18.00	15.70	16.80	-	-	-	-	+	-	+	+	-	-	-	+	-	-	-	+	-	+	-	-	+	+	+	4	3	4	-		5	1	1
37	13.20	14.25	14.20	15.60	-	-	-	+	+	-	-	+	-	-	+	+	-	-	-	-	-	+	-	-	+	+	+	1	1	1	-	22	1	1	1
41	16.25	17.95	15.10	15.65	-	-	+	-	-	+	-	+	-	-	-	+	-	-	-	+	+	-	-	+	-	-	+	2	4	6	-	1,6,13	6	2	1
42	14.30	14.80	15.25	15.45	-	-	+	-	+	+	-	+	-	-	+	-	-	-	-	+	-	-	-	+	-	+	+	4	3	4	-		4	3	1
43	15.85	17.10	15.50	16.50	+	+	-	-	+	-	-	+	-	-	-	-	-	-	-	+	+	-	-	+	-	-	+	3	5	2	+	36	7	3	2
44	18.55	19.10	17.35	19.00	+	-	-	-	+	-	-	+	-	-	-	+	-	-	-	-	-	+	-	-	+	+	+	5	8	11	-		7	1	1
46	15.65	15.70	14.70	16.15	-	-	-	-	+	-	-	+	-	-	-	+	-	+	-	-	+	-	-	-	+	+	+	1	3	1	-		1	1	1
48	15.75	17.35	14.95	16.25	-	+	-	+	+	-	-	+	-	-	+	+	+	-	+	-	+	+	-	-	+	+	+	3	2	0	-		2	1	2
49	14.50	16.20	14.70	15.80	-	-	-	-	+	-	-	-	-	+	-	+	-	+	-	-	+	-	-	-	+	-	-	1	1	1	+	27,42	1	1	6
50	15.35	15.65	15.30	16.35	+	-	-	-	+	-	-	+	-	+	-	+	-	+	-	-	-	-	-	-	-	-	+	1	1	1	-		1	1	3
52	13.95	15.80	15.65	16.30	-	-	-	-	+	-	-	+	-	+	-	+	-	+	-	-	+	-	-	-	-	-	+	1	1	1	-	10	1	1	3
54	14.60	16.25	15.10	16.10	-	+	-	-	+	-	-	+	-	-	+	-	-	+	-	-	+	-	-	-	-	+	+	1	1	1	-		9	4	2
56	16.90	18.00	15.25	15.90	-	-	-	-	+	-	-	-	-	-	-	+	-	+	-	-	+	-	-	-	+	-	-	1	1	1	-		1	1	4
58	15.70	16.90	13.90	16.25	-	+	-	-	+	-	-	+	+	-	+	-	-	+	-	-	+	-	-	-	+	+	+	1	1	1	-	10	9	4	2
63	16.60	18.20	17.70	18.35	+	-	+	-	-	-	-	+	-	-	+	-	-	+	-	-	+	-	-	+	-	-	-	2	1	1	-		7	3	1
64	14.85	16.45	17.40	18.25	-	-	-	-	+	-	-	+	-	-	-	+	-	-	-	+	+	-	-	-	+	+	+	2	4	6	-		8	1	1
71	13.75	16.20	15.05	16.25	-	-	-	-	+	-	-	+	-	-	+	+	-	+	-	-	+	-	-	+	-	+	+	1	1	10	-		3	2	1
84	15.30	16.10	15.05	16.45	+	-	-	-	-	-	-	+	-	-	-	+	-	+	-	-	+	-	-	+	-	+	+	2	3	10	-	35	6	2	1
86	15.90	16.70	13.60	14.70	-	-	+	-	+	-	-	+	-	+	-	+	-	+	-	-	+	-	-	-	+	+	+	9	5	13	-		7	1	3
87	19.60	22.10	16.10	17.20	-	-	-	-	+	-	-	+	-	-	-	+	-	-	-	+	+	-	-	-	+	+	+	7	4	6	-	6	7	1	1
88	15.50	18.05	15.40	15.60	+	-	-	-	+	-	-	+	-	-	-	+	-	-	-	+	-	-	+	-	+	+	+	3	6	1	+	16,17,26	2	1	1
89	15.80	16.75	17.10	17.55	-	-	-	-	-	-	+	+	-	-	-	+	-	+	-	-	+	-	-	-	+	-	+	1	1	1	+	31	1	1	1
92	16.40	16.60	13.95	15.75	+	-	-	+	+	-	-	+	-	-	+	+	+	-	+	-	+	+	-	-	-	-	-	3	2	0	-		2	1	1
94	15.75	17.05	16.20	15.85	-	-	-	-	+	-	-	+	-	-	-	+	-	+	-	-	+	-	-	-	-	-	-	1	1	1	-		1	1	1
101	10.55	10.70	14.70	15.15	+	-	+	-	+	+	-	+	-	-	+	-	-	-	-	+	-	-	-	+	-	+	+	4	3	3	-		4	3	1
106	14.35	16.15	15.50	16.45	-	-	-	-	-	-	-	+	+	-	+	+	+	+	-	-	-	+	-	+	-	+	+	8	1	1	-		7	2	1
108	14.95	16.55	15.40	16.70	+	-	-	-	+	-	-	+	-	-	+	-	-	-	-	+	+	-	-	-	+	+	+	6	6	0	-		7	1	1
110	15.55	16.50	16.20	16.80	-	-	-	+	+	-	-	+	-	-	+	+	-	+	-	-	+	-	-	-	+	+	+	1	3	1	+	33,35	1	1	1
116	13.90	17.65	17.45	17.15	-	-	-	-	+	-	-	+	+	-	-	+	+	-	-	+	-	-	+	-	+	+	+	4	1	12	-	42	5	1	1
117	16.10	18.45	17.90	18.45	+	-	-	-	+	-	-	+	+	-	+	+	-	-	+	-	+	-	+	-	-	+	+	3	2	0	-		2	1	1
Polymorphism frequency					0.32	0.17	0.13	0.15	0.77	0.06	0.06	0.89	0.09	0.13	0.36	0.85	0.13	0.55	0.11	0.26	0.70	0.13	0.06	0.28	0.06	0.72	0.45	0.66				0.15				

pleural bristle number, which suggests that different molecular variants in the *sca* region contribute to quantitative genetic variation in these two bristle traits.

Effects attributable to polymorphisms on the bristle traits were estimated for the nine diallelic polymorphic sites (Table 2); they ranged from 0.98 to 2.35 bristles for the main effect and from 0.39 to 1.06 bristles for the sex*site interaction effect. In additive genetic (σ_A) and phenotypic (σ_P) standard deviation units (Table 2), the main effect of the three diallelic polymorphic sites on abdominal bristle number averaged $1.48\sigma_A$ and $0.59\sigma_P$, and the main effect of the three sites on sternopleural bristle number averaged $1.39\sigma_A$ and $0.58\sigma_P$. The sex dimorphic effect of InF(−2.0) on abdominal bristle number was $0.95\sigma_A$ and $0.38\sigma_P$, and that of Eco RI(12.0)−1.8 and Eco RI(14.5) on sternopleural bristles averaged $0.54\sigma_A$ and $0.22\sigma_P$. The polymorphic sites associated with large phenotypic effects were not rare but occurred at intermediate frequencies. Although alleles with large effects were observed in some lines selected for extreme bristle number (*4*), these data are direct evidence that alleles with large effects on bristle number exist at intermediate frequencies in natural populations.

The amount of genetic variation in bristle number of the natural population that is attributable to the *sca* locus can be estimated assuming additivity of allelic effects. The additive genetic variance (σ_A^2) attributable to each significant polymorphism at *sca* is $0.5[1-\Sigma(p_i^2)]\ \sigma_c^2$ (*15*), where p_i is the frequency of the i^{th} class at a polymorphic site and σ_c^2 is the estimated variance component due to the main effect or to the site*sex interaction of each polymorphism. The estimated genetic variance associated with each of the 11 significant polymorphic sites is shown in Table 2. The genetic variance for the three polymorphic sites with sex-specific effects was negligible. The estimates of the genetic variance attributable to all other polymorphic sites were large, varying from 8 to 24% of the total second chromosome genetic variance of abdominal bristle number and from 7 to 11% of the total second chromosome genetic variance of sternopleural bristle number.

Because some of the polymorphic sites significantly associated with bristle number variation were in significant linkage disequilibrium with each other (Fig. 1), the overall genetic variance of both bristle traits contributed by *sca* was not equal to the sum of the estimated genetic variances associated with individual sites. We estimated the total genetic variance in bristle number contributed by the *sca* locus using only polymorphic sites associated with significant variation in bristle number that were not in linkage disequilibrium with each other. Haplotypes for each trait were constructed from these sites, and the genetic variance was estimated on the basis of these haplotypes. Taking into account the linkage disequilibria among sites and excluding the three sites associated with only sex-specific effects, three sites were used to construct haplotypes associated with variation in abdominal [Eco RI(6.5), Pst I(26.6), and SSCP 1447] and sternopleural [Pst I(−12.0), Eco RI(−1.5), and InL(5.3)] bristle number (Table 1). The genetic variance attributable to abdominal and sternopleural bristle haplotypes was estimated as for individual sites (*15*), pooling all unique haplotypes into a single class (Table 2). The overall genetic variance in abdominal and sternopleural bristle number contributed by the *sca* locus was 0.388 [AB(1) haplotype; 0.126 for the AB(2) haplotype, constructed without SSCP 1447] and 0.132, respectively, or 32% [AB(1) haplotype; 10% for AB(2) haplotype without SSCP 1447] and 21%, respectively, of the second chromosome genetic variance of the two traits. The second chromosome contains ~40% of the *D. melanogaster* genome; thus, the estimated contribution of the *sca* locus is 13 and 8% of

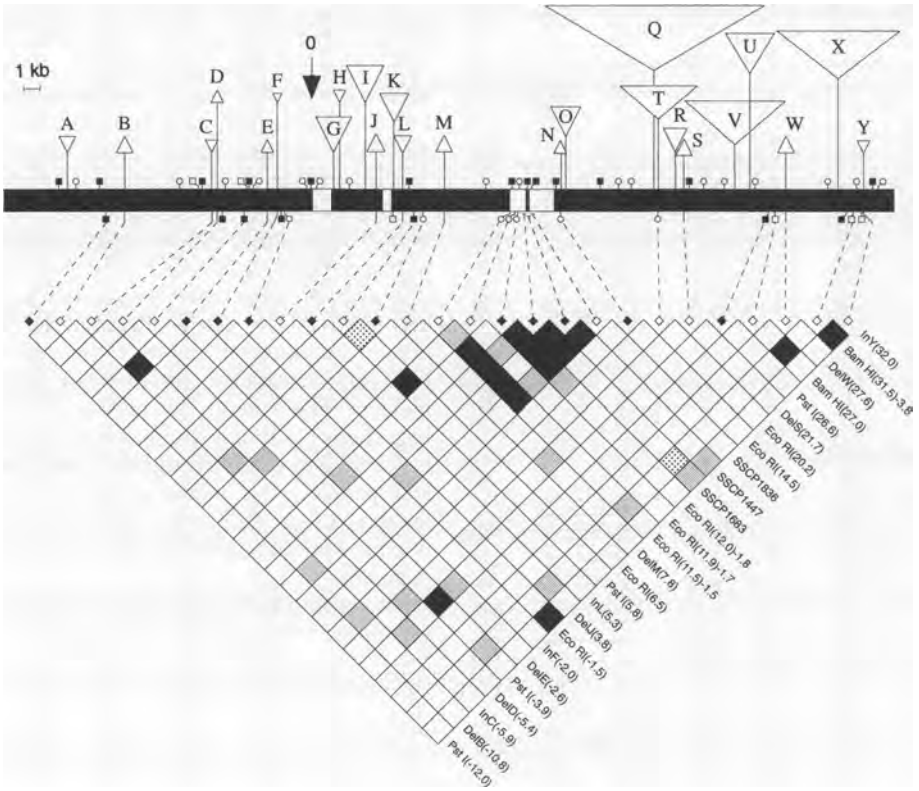

Fig. 1. (**Upper**) Restriction map variation in the *scabrous* locus region of *D. melanogaster* among 47 second chromosomes. DNA was isolated from adult flies from each homozygous chromosome line and digested separately with three restriction enzymes (Pst I, Bam HI, and Eco RI). Southern (DNA) blot hybridization was done with nine probes that completely covered the 45-kb region. The probes were labeled with digoxigenin-dUTP (Boehringer Mannheim) (*26*). Polymorphic sites of the restriction enzymes are shown below the map. Monomorphic sites of the restriction enzymes and insertion or deletion polymorphisms are indicated above the map. Solid squares, Pst I; open squares, Bam HI; and open circles, Eco RI. The open portions of the map represent the *sca* coding regions, with the 5′ end to the left, the 3′ end to the right, and the start of transcription at 0 (indicated by the arrow). The approximate sizes (in kilobases) of insertions (In) and deletions (Del) are as follows: A, 0.2; B, 0.2; C, 0.1; D, 0.1; E, 0.1; F, 0.05; G, 2.0; H, 0.1; I, 2.3 with an Eco RI site; J, 0.3; K, 1.3; L, 0.2; M, 0.2; N, 0.1; O, 1.0; Q, >10; R, 0.6; with an Eco RI site; S, 0.4; T, 3.2; U, 1.9 with an Eco RI site; V, 3.9 with an Eco RI site; W, 0.2; X, 4.9 with an Eco RI site; and Y, 0.1. Single-stranded conformation polymorphism (SSCP) was investigated for three DNA fragments (*9*): 1683 (186 nucleotides), 1447 (300 nucleotides), and 1836 (290 nucleotides), which correspond to the region of the third intron and parts of the third and fourth exons. (**Lower**) Pairwise linkage disequilibria among the 27 polymorphisms with frequencies between 3/47 and 44/47. The name of each of these sites is listed diagonally and their corresponding map positions are indicated by the dashed lines. Significance levels are depicted by shading: black, $P < 0.001$; dot shading, $P < 0.01$; light shading, $P < 0.05$; and no shading, $P > 0.05$. Solid diamonds indicate the 11 polymorphic sites that show significant association with variation in bristle number. Among them, DelE(−2.6) is in significant ($P < 0.05$) linkage disequilibrium with Eco RI(12.0)−1.8 and Pst I(26.6) and is almost in significant (Fisher's exact test = 0.054) linkage disequilibrium with Eco RI(6.5); alleles at SSCP 1447 and SSCP 1683 are in strong ($P < 0.001$) linkage disequilibrium with each other; Eco RI(12.0)−1.8 is in significant ($P < 0.05$) linkage disequilibrium with Eco RI(14.5).

the total genetic variance for abdominal bristle number and sternopleural bristle number, respectively.

The relation between the constructed haplotypes and the genetic variance in bristle number among the 47 chromosome lines is shown in Fig. 2. The mean bristle number of each chromosome line was plotted against its rank; the haplotype of each chromosome based on Eco RI(6.5) and Pst I(26.6) for abdominal bristle haplotype (Fig. 2A) and on Pst I(−12.0), Eco RI(−1.5), and InL(5.3) for sternopleural bristle haplotype (Fig. 2B) is also indicated. The most common haplotypes for each trait were distributed to one side of the curve, whereas the remaining haplotypes clustered together at the other side.

These results have important implications with regard to the number of loci and effects and frequencies of alleles causing naturally occurring quantitative variation. Quantitative genetic variation is generally thought to be caused by many loci, alleles at each of which have small effects on the traits (*2*, *16*). We have now shown that ~10% of the total genetic variation in abdominal and sternopleural bristle number can be attributed to variation at the *sca* locus; previously, 5% of the genetic variation in these traits was associated with insertional variation in *ASC* (*1*). Thus, the infinitesimal model (many loci, alleles at each of which have small effects on traits) is not an accurate reflection of the genetic basis of quantitative variation in bristle number. These results further support the hypothesis (*1*) that a strong correlation exists between loci identified as important and specific in developmental genetic studies and those contributing most to the phenotypic variation for that trait in natural populations.

In contrast to the situation in *ASC* (*1*), we observed that diallelic polymorphisms at

Table 2. Polymorphic sites significantly associated with bristle number variation and estimates of the associated genetic variation in a natural population, assuming additivity of allelic effects. Asterisks indicate a polymorphic site with a significant sex*site interaction effect but not a significant main effect. The differences between the means of two classes of diallelic polymorphic sites were estimated as $|X_{(+)} - X_{(-)}|$ for main effects, where $X_{(+)}$ and $X_{(-)}$ are the means of the chromosome lines in which the polymorphism is present (+) or absent (−), respectively. The sex*site interaction effects were estimated as $|(X_{+M} - X_{-M}) - (X_{+F} - X_{-F})|$, where $(X_{+M} - X_{-M})$ is the difference in males and $(X_{+F} - X_{-F})$ is the difference in females. SE is the standard error of $|X_{(+)} - X_{(-)}|$. Unit (σ_P) is $|X_{(+)} - X_{(-)}|$ expressed as a fraction of the phenotypic standard deviation (σ_P). The additive genetic variance (σ_A^2) of each site was estimated with the formula derived in (*15*). Unique SSCP classes and haplotypes were pooled into one class or one haplotype when estimating additive genetic variance (Table 1). The percentage of the total genetic variance of the second chromosome explained by the estimated σ_A^2 is also shown. The additive genetic (σ_A) and phenotypic (σ_P) deviations are the square roots of the additive genetic and phenotypic variance of bristle number for the second chromosome (*7*) and are respectively 1.11 and 2.77 for abdominal bristles and 0.80 and 1.91 for sternopleural bristles.

Polymorphic site	*P* value of *F* test	Effect $[X_{(+)} - X_{(-)}]$			Additive genetic variance	
		Absolute	SE	Unit (σ_P)	σ_A^2	%
		Abdominal bristle number				
DelE(−2.6)	0.014	2.35	0.45	0.85	0.140	11
InF(−2.0)*	0.043	1.06	0.46	0.38	0.006	0
Eco RI(6.5)	0.026	1.47	0.22	0.53	0.112	9
Pst I(26.6)	0.038	1.10	0.15	0.40	0.094	8
SSCP 1447	0.004				0.299	24
SSCP 1683	0.018				0.209	17
AB(1) haplotype					0.388	32
AB(2) haplotype					0.126	10
		Sternopleural bristle number				
Pst I(−12.0)	0.030	0.98	0.10	0.51	0.055	9
Eco RI(−1.5)	0.018	1.31	0.12	0.68	0.068	11
InL(5.3)	0.042	1.04	0.11	0.55	0.047	7
Eco RI(12.0)−1.8*	0.019	0.47	0.19	0.24	0.004	1
Eco RI(14.5)*	0.039	0.39	0.19	0.20	0.003	0
SB haplotype					0.132	21

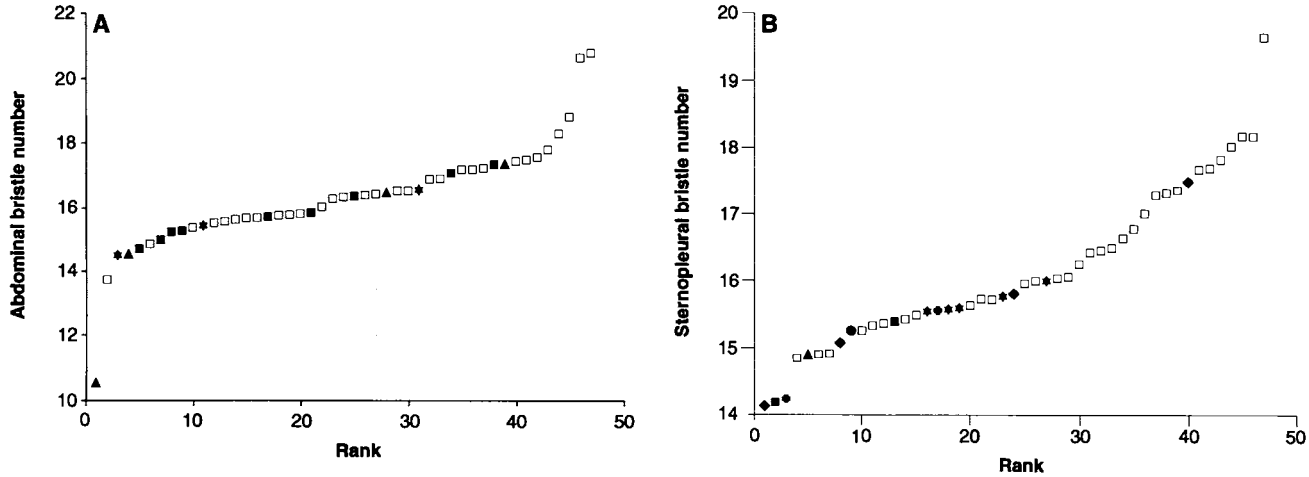

Fig. 2. Association of haplotypes with bristle number variation. (**A**) The correlation of the AB(2) haplotype (derived from the independent polymorphic sites with significant effects on abdominal bristle number (Table 1) with abdominal bristle number. The mean abdominal bristle number of each chromosome line is plotted against its rank. The haplotype of the chromosome is indicated [in the order of Eco RI(6.5) and Pst I(26.6)] as: open squares, +−; solid squares, ++; solid triangles, −+; and solid stars, −−. The outside gray (dashed) lines indicate the mean bristle number of the common haplotype on the ordinate and the median bristle number of the common haplotype on the abscissa; the inner gray lines indicate the mean and the median of the remaining haplotypes. (**B**) Plotted as (A) but for the SB haplotype (but here without pooling the two unique haplotypes) (Table 1). The haplotype of the chromosome is indicated [in the order of Pst I(−12.0), Eco RI(−1.5), and InL(5.3)] as: open squares, −+−; solid stars, +++; solid diamonds, −++; solid crosses, −−−; solid squares, +−−; solid triangle, +++; and solid circles, −−+.

the *sca* locus are associated with large phenotypic effects, segregating at intermediate frequencies (*17*). If a few loci account for a substantial fraction of genetic variation in a quantitative trait, allelic effects at these loci must be large. Segregation of alleles with large effects on quantitative traits, at low frequencies, is predicted by theoretical models in which the equilibrium distribution of quantitative variation reflects a balance between the input of new mutations affecting the trait and their elimination either by stabilizing selection on the trait (*16*) or because the mutations have deleterious pleiotropic effects on fitness (*18*). Our observation is consistent with maintenance of quantitative genetic variation by mutation-selection balance only if some mutations of large effect at the *sca* locus are selectively neutral (*19*); however, most evidence suggests that mutations that affect bristle number have deleterious pleiotropic effects on fitness (*20*). For statistical reasons, we could not include rare polymorphic sites in our analysis and, therefore, cannot exclude the possibility that rare *sca* alleles with large effects also contribute a significant fraction of the genetic variation in bristle number. These results indicate that quantitative genetic variation caused by alleles of large effect at a few loci, segregating at intermediate frequencies, should be accommodated in models for the maintenance of quantitative genetic variance. The presence of alleles with large effects at intermediate frequencies in natural populations may also be relevant to ongoing controversy concerning the nature of genetic differences in morphological traits between closely related species (*21*).

Several unknown factors could potentially bias our estimates of the magnitude of genetic variation in bristle number associated with molecular polymorphisms at *sca*. In accord with previous observations (*22*), we have assumed strictly additive allelic effects, but dominance can either increase or decrease the estimated genetic variance (*23*). The estimates are biased upward if some of the significant associations are spurious. Finally, the observed polymorphic sites may not be the direct causes of the differences in bristle number; instead, they may be in linkage disequilibrium with the actual molecular variants causing the effects. Such an indirect association would result in underestimates of the effects and hence of the genetic variance (*24*). However, because there is little linkage disequilibrium among sites >2 kb apart in the 45-kb region surrounding *sca* (Fig. 1), it is unlikely that association of bristle number variation with polymorphisms at *sca* is attributable to linkage disequilibrium between polymorphisms at *sca* and variation at other loci.

The observation that the sites associated with variation in abdominal bristle number differ from those associated with variation in sternopleural bristle number may reflect the intricate regulation of expression of *sca* (*5*, *25*). For example, the third intron and fourth exon are proposed as regulatory regions with binding sites for the homeobox transcription factors encoded by *Ubx*, *abA*, and *AbdB* (*25*). These regions may participate in negative regulation of *sca* expression in abdominal segments. Indeed, we detected polymorphic sites (SSCP 1447 and SSCP 1683) in this region that were strongly associated with variation in abdominal bristle number. A more mechanistic interpretation of the impact of *sca* on quantitative genetic variation will require further molecular and developmental studies and more focused analyses of specific alleles from natural populations.

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6. Forty-seven independent second chromosomes extracted from flies caught at Raleigh Farmer's Market in May 1988 were substituted into a Samarkand *X; ry*^{506} chromosome 3 isogenic genetic background. The homozygous effect of each chromosome on abdominal and sternopleural bristle numbers was estimated from 10 male and 10 female flies scored in each of two replicate vials. All the flies were reared at 25°C on 10 ml of cornmeal-agar-molasses medium in shell vials.
7. The additive genetic variance (σ^2_A) of the second chromosome equals one-half of the variance component due to homozygous chromosome lines, when strict additivity is assumed (*2*). Variance components were estimated from the linear model, $Y_{jklm} = u + S_j + L_k + (S^*L)_{jk} + R(L)_{l(k)} + S^*R(L)_{jl(k)} + e_{jklm}$, where Y_{jklm} is the bristle number of individual m, of the j^{th} sex, the k^{th} line, and the l^{th} replicate; j = 1 and 2, l = 1 and 2, k = 1, 2, . . . 47, and m = 1, 2, . . . 10; u is the overall mean. Variance was partitioned into sources due to the independent fixed effects of sex (S_j), the random effects of chromosome line (L_k), sex*line interaction [$(S^*L)_{jk}$], replicates nested within line [$R(L)_{l(k)}$], sex*replicate interaction nested within line [$S^*R(L)_{jl(k)}$], and error (e_{jklm}). The estimate of additive genetic variance of the second chromosome was 1.23 for abdominal bristle number and 0.64 for sternopleural bristle number. The phenotypic variance was 7.678 for abdominal bristle number and 3.631 for sternopleural bristle number. With the above model, the additive genetic covariance between abdominal and sternopleural bristle number was estimated as 0.39, and the genetic correlation coefficient was $0.39/[\sqrt{(1.266 \times 0.64)}] = 0.43$ (*2*). All statistical analyses were done with SAS programs (SAS Institute, Cary, NC).
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9. SSCP for the three DNA fragments indicated in Fig. 1 was investigated as described [M. Aguadé *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **91**, 4658 (1994)]. For SSCP 1447, the primers were 5′-TGGATCGATTGCAGTCGCTGGTC-3′ and 5′-TCTGATGATGTGGTCGCTTGTGC-3′; for SSCP 1683, the primers were 5′-GTTCGCCAATGCTCAGATCACCTCG-3′ and 5′-GACCAGCGACTGCAATCG-3′; and for SSCP 1836, the primers were 5′-TCACCCACCTGAACAAGC-3′ and 5′-AGTCTGCACTGCCATCGAACCG-3′. Primers were designed on the basis of the published sequences (*5*). The entire third intron was sequenced and determined to be 199 nucleotides.
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12. The *P* values associated with given *F* statistics do not accurately reflect the probability of a positive association between molecular and genetic variation for sites with a frequency of <3 out of 47. This was demonstrated by generation of the lesser of 1000 or all possible dummy molecular data sets for polymorphic site frequencies of 2, 3, 4, 5, and 7; reanalyzing each data set and calculating *F* statistics, and then comparing the observed and expected distribution of the *F* statistics. We therefore focused our analysis on 27 polymorphisms with frequencies of 3/47 to 44/47—24 restriction site and length polymorphisms with frequencies of 3/47 to 44/47—24 restriction site and length polymorphisms and three SSCP loci—as well as the presence or absence of large insertions of >0.5 kb (Table 1). All rare alleles (<3 out of 47) of multiple-allelic SSCP loci were pooled into single classes; that is, SSCP 1447 alleles 5, 6, 7, 8, and 9 were pooled into class 5; SSCP 1683 alleles 5, 6, and 8 were pooled into class 5; and SSCP 1836 alleles 2, 4, 7, 8, 9, 10, 11, 12, and 13 were pooled into class 2.
13. The following linear model was used to test whether individual polymorphic sites at the *sca* region were associated with variation in bristle number: $Y_{ijklm} = u + M_i + S_j + (M^*S)_{ij} + L(M)_{k(i)} + S^*L(M)_{jk(i)} + R(L^*M)_{l(ki)} + S^*R(L^*M)_{jl(ki)} + e_{ijklm}$. The notation for each variable is described in (*7*), with the addition of the variable *M*, representing the polymorphic site tested, where i = 1 and 2 for diallelic polymorphic sites and i = 1, 2, 3, . . . r for SSCP loci and derived haplotypes, where r is the number of alleles (classes) of a polymorphic site. All effects were considered random, except sex, polymorphic site, and their interaction (sex*site interaction). *F* tests were conducted by dividing the mean square (MS_M) for polymorphic site by the mean square ($MS_{L(M)}$) of lines nested within the polymorphic site for the main effect, and the mean square (MS_{S^*M}) of sex*site interaction by the mean square ($MS_{S^*L(M)}$) of sex*line interaction within the site for the sex*site interaction effect.
14. The random permutation test we used here is more appropriate than the sequential Bonferroni test [S. Holm, *Scand. J. Stat.* **6**, 65 (1979)] with respect to the hypothesis and nonindependent polymorphisms we tested. None of the polymorphisms was significant by the conservative sequential Bonferroni test.
15. The additive genetic variance (σ^2_A) associated with each polymorphic site was estimated from the variance component due to the main effect or the sex*site interaction of a polymorphic site. The variance component due to the main effect of a polymorphic site is given by $\sigma_c^2 = [MS_M - MS_{L(M)}]/NJ$, where N is the number of observations per chromosome line; J, which equals $1/(r-1)[n - \Sigma(J_i)^2/n]$, is the standardized coefficient when there is an unequal number of lines (J_i) for each class i of a polymorphic site; r is defined as in (*13*); n is the total number of chromosome lines; and MS_M and $MS_{L(M)}$ are as in (*13*). Because $MS_M = N/(r-1)\ \Sigma\{J_i[X_i -$

$\Sigma\{p_iX_i)]^2\}$, where p_i and X_i are the frequency and the mean, respectively, of chromosome lines homozygous for class i of a polymorphic site, and $J_i = np_i$, then $\Sigma\{p_i[X_i - \Sigma(p_iX_i)]^2\} = [1 - \Sigma(pi)^2][\sigma_c^2 + MS_{L(M)}/(NJ)]$. To account for the variance ($MS_{L(M)}$) due to the chromosome line effect and sampling error, $\Sigma\{p_i[X_i - \Sigma(p_iX_i)]^2\} = [1 - \Sigma(p_i)^2]\,\sigma_c^2$. Assuming strictly additive allelic effects, the additive genetic variance associated with a polymorphic site of r classes (that is, r alleles at one locus) is $\sigma_A^2 = 0.5\,\Sigma\{p_i[X_i - \Sigma(p_iX_i)^2\}$ [O. Kempthorne, *Introduction to Genetic Statistics* (Wiley, New York, 1957), pp. 318–324]. With the above relation, we can show $\sigma_A^2 = 0.5\,[1-\Sigma(p_i)^2]\,\sigma_c^2$, where σ_c^2 was estimated on the basis of the model described in (*13*). The same procedure was used to estimate the additive genetic variance associated with haplotypes and the sex*site interaction effect of a polymorphic site.

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17. At *ASC*, large insertions (>0.5 kb) as a class were associated with quantitative variation in bristle number (actually, an average reduction in bristle number), but each insertion was individually rare. The class of large insertions in the *sca* region was not significantly associated with bristle number variation among the second chromosome lines. The contrast in the nature of the molecular variants associated with phenotypic variation in these two regions may reflect an inherent difference in the loci or may be a consequence of the reduction in the amount of DNA sequence polymorphism (not insertional polymorphism) found in regions of low cross-over frequency per physical length [M. Augadé *et al.*, *Genetics* **122**, 607 (1989)].
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23. With complete dominance, the genetic variance associated with a diallelic polymorphic site is given by $\sigma_G^2 = 2q(1 + q)\sigma_A^2$, where q is the gene frequency of the recessive allele and σ_A^2 is the additive genetic variance (*2*). For a given gene frequency and effect at a diallelic locus, the minimal variance occurs when the recessive allele has the lower frequency, and the maximal variance occurs when the recessive allele has the higher frequency. For example, estimates of genetic variance in abdominal bristle number attributable to *Eco* RI(6.5) polymorphism with complete dominance range from 0.038 to 0.35 (compared to 0.112, assuming strict additivity). For the range of gene frequencies and effects observed, the estimated variance, assuming additivity, could be over- or underestimated by a factor of 3.
24. The correlation between the effect (a) attributable to a polymorphic site and the effect α due to the actual molecular variant with which the site is associated is given by $\alpha = \{[p(1-p)]/D\}a$, where p is the frequency of the polymorphic site and D is the linkage disequilibrium between the molecular variant directly causing the bristle number effect and the polymorphic site associated with it. Because $D \leq p(1-p)$, then $\alpha \geq a$; that is, the estimated effect attributable to a polymorphic site is an underestimate of the effect of the actual variant with which the site is associated.
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5 July 1994; accepted 29 September 1994.

RNA14 and RNA15 Proteins as Components of a Yeast Pre-mRNA 3′-End Processing Factor

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Most eukaryotic pre–messenger RNAs are processed at their 3′ ends by endonucleolytic cleavage and polyadenylation. In yeast, this processing requires polyadenylate [poly(A)] polymerase (PAP) and other proteins that have not yet been characterized. Here, mutations in the *PAP1* gene were shown to be synergistically lethal with previously identified mutations in the *RNA14* and *RNA15* genes, which suggests that their encoded proteins participate in 3′-end processing. Indeed, extracts from *rna14* and *rna15* mutants were shown to be deficient in both steps of processing. Biochemical complementation experiments and reconstitution of both activities with partially purified cleavage factor I (CF I) validated the genetic prediction.

The pre-mRNAs of the yeast *Saccharomyces cerevisiae* are processed at their 3′ ends to produce transcripts ending with a poly(A) tail of about 70 adenosine residues [reviewed in (*1*)]. Yeast RNA 3′-end formation has been reconstituted in vitro (*2*) and shown to be similar to that in mammals (*3*). It consists of two reactions: endonucleolytic cleavage of the pre-mRNA and subsequent polyadenylation of the upstream fragment. Correct cleavage of the precursor occurs in vitro by combination of two partially purified factors, CF I and CF II, whereas polyadenylation of the upstream fragment requires CF I, polyadenylation factor I (PF I), and PAP itself (*4*). Thus, CF I is required for both steps of processing. Thus far, only the gene coding for PAP has been identified and shown to be essential for cell viability (*2*, *5*).

A selective screen for *S. cerevisiae* mutants that are sensitive to cordycepin (3′-deoxyadenosine) at 22°C and are temperature-sensitive at 37°C has revealed two genetically independent mutants called *rna14* and *rna15* (*6*). In these mutants, the steady-state concentration of the polyadenylated mRNAs was shown to rapidly decrease at the nonpermissive temperature (37°C), with a shortening of the poly(A) tails. This was also observed with a well-known RNA polymerase II mutant, *rpb1-1*. Because transcriptional activity was not substantially altered in *rna14* and *rna15*, these phenotypes have been interpreted as an impairment of mRNA stability (*6*). However, a defect in the maturation of pre-mRNA 3′ ends could also explain the mutant phenotypes. The latter explanation would mean that no new mature mRNA is produced, which is consistent with the phenotypes observed. Genetic and biochemical evidence is provided in this report that strongly supports the involvement of RNA14 and RNA15 proteins in 3′-end processing of pre-mRNAs; more precisely, as components of CF I.

If *RNA14*- or *RNA15*-encoded proteins are involved in the maturation of pre-mRNA 3′ ends, they may physically or functionally interact with PAP. This interaction can be assayed genetically through the analysis of double mutants of their genes: The combination of these mutations may show a synergistic interaction and lead to cell death under any conditions of growth (*7*). We thus introduced *rna14-1* or *rna15-1* mutations into a strain carrying a chromosomal disruption of the *PAP1* gene, rescued by the p*PAP1* plasmid, which contains the wild-type *PAP1* gene and the *URA3* marker. These strains (*8*) were then transformed with an *ADE2*-marked plasmid carrying the temperature-sensitive allele *pap1-5* (p*Apap1-5*) (*9*). Because of the presence of the *rna14-1* or *rna15-1* alleles, these strains are strictly temperature-sensitive but can grow at the permissive temperature (24°C) on a selective medium that allows the maintenance of the plasmids (Fig. 1A, sectors 1 and 6). However, the strains were inviable once the p*PAP1* plasmid was lost on selective medium containing 5-fluoroorotic acid (5-FOA) (*10*), even at the low temperature (Fig. 1B, sectors 1 and 6). Because on this medium the essential functions of *RNA14*, *RNA15*, and *PAP1* genes were only provided by their respective mutant alleles, we concluded that the observed lethality was due to the enhancement of the conditional defect that each of the mutants carries on its own. This conclusion was strengthened by the fact that the synergistic lethality was reversed when we introduced the plasmid-borne *RNA14* wild-type gene into the *rna14-1 pap1-5* mutant context but not when we introduced the *RNA15* gene (Fig. 1B, compare sectors 2 and 3, respectively). For the same reasons, an *RNA15*-containing plasmid allowed the *rna15-1 pap1-5* mutant to grow on 5-FOA medium, whereas transformation with the *RNA14* gene did not (Fig. 1B,

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