LONG PALPI, AN AUTOSOMAL, SEX LIMITED CO-DOMINANT MUTANT IN *CULEX TRITAENIORHYNCHUS*

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ABSTRACT. A new mutant long palpi (*Lp* was discovered following treatment with ethylmethanesulfonate. The mutant is co-dominant to the wild type allele, sex limited and is controlled by a locus in linkage group III. Homozygous *Lp* females which exhibit the longest palpal type also have reduced viability, do not readily mate, or lay eggs. Heterozygous *Lp* females have palpi intermediate in length and show normal viability and fertility. Males heterozygous and homozygous for *Lp* have normal palpi and are fertile. In crosses with three other linked loci the gene sequence was *Lp-Adh-bw-ae* and the recombination frequencies were as follows: *Lp-Adh*=22.5%, *Lp-bw*=38.6% and *Lp-ae*=50.3%. Thus *Lp* represents the current known terminal marker on linkage group III, now over 50 map units in length.

During an experiment to induce temperature-sensitive lethals with ethylmethanesulfonate in *Culex tritaeniorhynchus* Giles several new mutants were found (Sakai and Baker 1974). One mutant, characterized by the elongation of the female palpi (Fig. 1), has been named *Long palpi* (*Lp*). *Lp* appears to be sex limited (only the female palpi and not the male are affected) and co-dominant, as two distinct types of *long palpi* are found in females. The palpi of heterozygous *Lp* individuals are of intermediate length (Fig. 1B) compared to wild type (Fig. 1A) and the presumptive homozygotes, *Lp/Lp*, which extend to the light band on the proboscis (Fig. 1C). There is little variability of these two types and no overlap. As will be described later, the presumptive *Lp* homozygous females appear to be sterile as repeated efforts to obtain progeny have been unsuccessful. Mating tests suggest that homozygous males are fertile but do not show the mutant character. The mutant is maintained by backcrossing *Lp* females with sib males and culling out females with normal palpi each generation. This paper reports the results of genetic experiments with *Lp* and presents evidence that *Lp* is under the control of a locus in linkage group III. Five loci, *cl* (curved leg), *Adh* (alcohol dehydrogenase), *s* (straw larva), *bw* (brown eye) and *ae* (abnormal eye) have been previously reported for linkage group III (Baker and Sakai 1973; Sakai et al. 1973, 1976), and the current linkage map is as follows:

cl	Adh	s	bw	ae
0	12.9	20.5	32.9	47.5

MATERIALS AND METHODS

The following strains of mosquitoes were used in the crosses:

1) *bw ae*—this strain carried the markers brown eye and abnormal eye (Sakai and Baker 1976).
2) *Lp*—this strain carried only the long palpi mutant.
3) *Lp bw ae*—this strain carried long

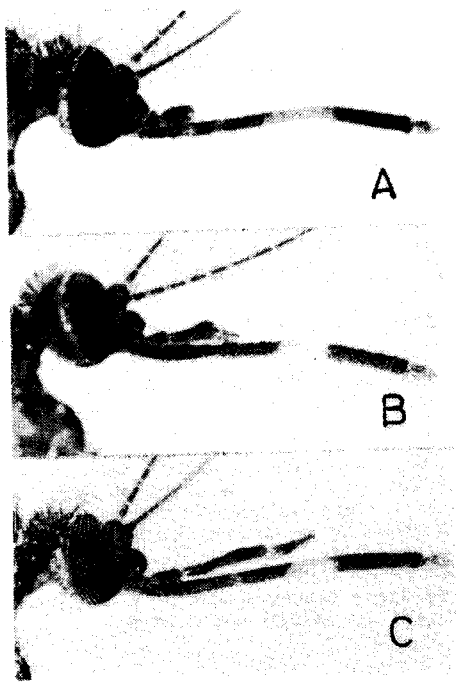

Fig. 1. *Culex tritaeniorhynchus* females. A—wild type palpi (±/±); B—heterozygous long palpi (*Lp*/±); C—homozygous long palpi (*Lp*/*Lp*).

palpi, brown eye and abnormal eye.

4) *Lp Adh-C ae*—this strain carried long palpi and abnormal eye and in addition, the C variant of alcohol dehydrogenase (Sakai *et al.* 1973).
5) *Adh-E*—a wild type strain homozygous for the E electrophoretic variant of alcohol dehydrogenase.
6) *Adh-C ae*—a strain homozygous for *Adh-C* and abnormal eye.

Mass matings were made for all crosses but egg rafts were isolated and reared as individual families (Sakai et al. 1976).

Since *Lp* is not expressed in males and homozygous *Lp* females are sterile, it was impossible to distinguish in some parental and backcrosses whether or not the father was carrying the *Lp* chromosome. In these crosses, those rafts that did not show long palpi females were excluded from the data. The larvae were fed liver powder daily. The alcohol dehydrogenase phenotypes were determined as previously described (Sakai et al. 1973).

RESULTS AND DISCUSSION

CROSSES INVOLVING *Lp-bw-ae*. A summary of the results of crosses showing the relationship among *Lp*, *bw* and *ae* and the chi square analysis is given in Tables 1 and 3 respectively. In the reciprocal parental crosses (A-D) no significant departures were found from the expected 1:1 ratio for long palpi and wild type palpi nor for sex. These matings confirm that *bw* and *ae* are recessive and suggest that *Lp* is dominant and sex-limited (only females show the trait).

In backcrosses involving heterozygous females (E-H), no significant departures from the 1:1 ratio were observed for sex, +:*Lp*, +:*bw* or +:*ae*. Absolute linkage was observed among *Lp*, *bw* and *ae* indicating that *Lp* is in the third linkage group and confirming the general absence of crossing over in females of *C. tritaeniorhynchus* (Baker and Rabbani 1970). In the heterozygous male backcrosses (I-L) some significant and highly significant departures were observed between allelic alternatives. As in previous experiments (Sakai *et al.* 1976), *bw* and *ae* frequently showed reduced viabilities when compared to the wild type. The chi squares testing for independent assortment between *Lp* and *ae* (Table 3) generally confirm the presence of linkage among the three markers. The only exceptions were the chi squares testing independent assortment between *Lp* and *ae* in crosses J and K. Table 4, which summarizes the crossover frequencies among the markers, indicate that *Lp* and *ae* are the two extreme distal loci and assort nearly independently of each other. Thus, it is not surprising that some of the chi squares are not significant. The gene sequence appears to be *Lp-bw-ae*.

CROSSES INVOLVING *Lp-Adh-ae*. Table 5 summarizes the crosses (O-R) showing the relationship of *Lp* to *Adh* and *ae*. As *Lp*

Table 1. Summary of crosses showing the reltaionship among *Lp*, *bw* and *ae*.

Cross	Proposed Parental Genotype ♀		♂	f*	♀ +	♀ bw	♀ ae	♀ bw ae	♀ Lp	♀ Lp bw	♀ Lp ae	♀ Lp bw ae	♂ +	♂ bw	♂ ae	♂ bw ae
A	Lp bw ae / + bw ae	x	+ + + / + + +	5	102	10	0	0	111	0	0	0	224	0	0	0
B	+ + + / + + +	x	Lp bw ae / + bw ae	6	85	0	0	0	99	0	0	0	194	0	0	0
C	Lp + + / + + +	x	+ bw ae / + bw ae	5	152	0	0	0	134	0	0	0	281	0	0	0
D	+ bw ae / + bw ae	x	Lp + + / + + +	7	189	0	0	0	172	0	0	0	388	0	0	0
E	Lp bw ae / + + +	x	+ bw ae / + bw ae	5	84	0	0	0	0	0	0	77	87	0	0	84
F	+ + + / Lp bw ae	x	+ bw ae / + bw ae	5	63	0	0	0	0	0	0	58	66	0	0	50
G	Lp + + / + bw ae	x	+ bw ae / + bw ae	3	0	0	0	78	94	0	0	0	72	0	0	70
H	+ bw ae / Lp + +	x	+ bw ae / + bw ae	5	0	0	0	91	112	0	0	0	117	0	0	117
I	+ bw ae / + bw ae	x	Lp bw ae / + + +	16	177	7	29	112	130	18	7	159	278	33	49	264
J	+ bw ae / + bw ae	x	+ + + / Lp bw ae	8	108	4	14	67	62	11	3	74	138	22	15	108
K	+ bw ae / + bw ae	x	Lp + + / + bw ae	10	69	22	3	75	107	1	23	45	181	13	24	112
L	+ bw ae / + bw ae	x	+ bw ae / Lp bw ae	10	82	11	6	88	100	13	14	37	155	20	26	149

* f indicates number of families examined.

appears to be sex-limited, only females were scored in this experiment. The heterozygous female backcrosses (O and P) confirm the linkage among *Lp, Adh* and *ae* (Table 3). The male backcrosses (Q and R) indicate that the gene sequence is *Lp-Adh-ae*. The combined data from all the heterozygous male backcrosses (I-L and Q-R) give the following order: *Lp-Adh-bw-ae*. The observed recombination frequencies are: *Lp-Adh*=22.5%, *Lp-bw*=38.6%, *Adh-ae*=29.1% and *bw-ae*=11.1%. Thus, the length of the third linkage group is now approximately fifty map units, with *ae* as one terminal marker and *Lp* the other.

HOMOZYGOSITY FOR *Lp*. No true-breeding strain of *Lp* has been isolated even after 25 generations of selection. This suggested that homozygosity for *Lp* was either lethal or sterile. A series of experiments were done to investigate this phenomenon. Since the mutant is not expressed in males, a mating scheme (Fig. 2) was necessary to ensure that a particular male was heterozygous for *Lp*. *Lp* females were back-crossed to *bw ae* males for two generations: All of the resulting wild type males should carry *Lp*. These *Lp* males (*Lp*++/+*bw ae*) were then crossed to *Lp* females (*Lp*++/+++). The phenotypes of the offspring from 20 families were as follows:

wild type palpi ♀ ♀	= 288
long palpi ♀ ♀	= 535
extra long palpi ♀ ♀	= 70
wild type palpi ♂ ♂	= 953
Total	=1846

There was a large deficiency in the recovery of extra long palpi females, but the ratio of wild type palpi females to long palpi females fit an expected 1:2 (chi square=1.02; 0.5>p>0.3). Perhaps some

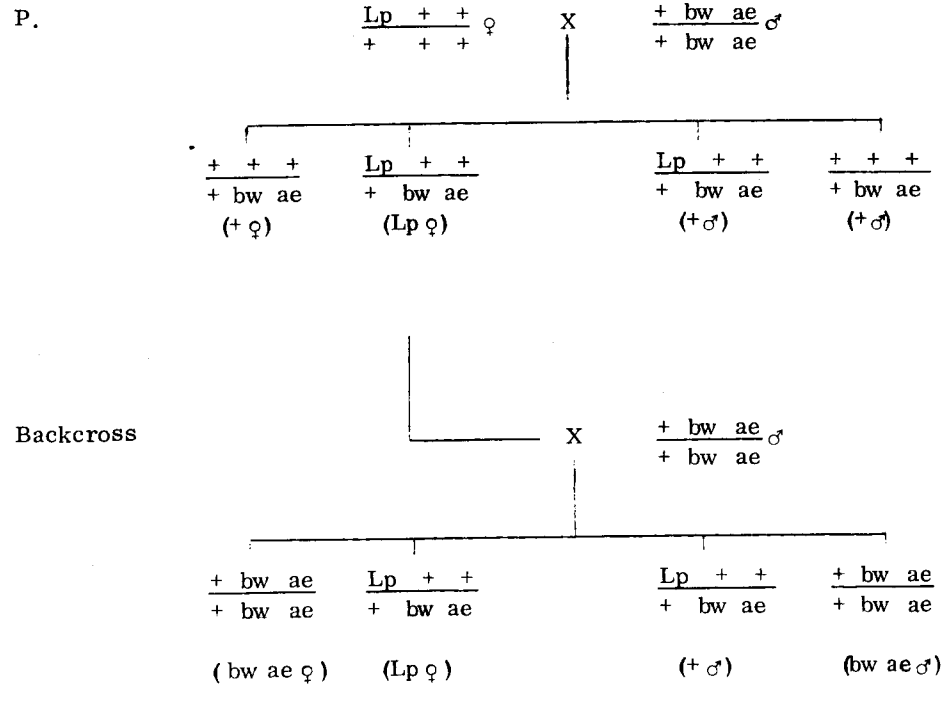

Fig. 2. Scheme to synthesize males heterozygous for *Lp*. Although *Lp* is not expressed in males all resulting + ♂ ♂ from the backcross should have the *Lp* chromosome.

Table 2. Summary of crosses showing the relationship among *Lp*, *Adh* and *ae* in female offspring.

Cross	Proposed Parental Genotype ♀	Proposed Parental Genotype ♂	f*	+ CE	+ C	ae CE	ae C	Lp CE	Lp C	Lp ae CE	Lp ae C
M	Lp Adh-C ae / + Adh-C ae	+ Adh-E + / + Adh-E +	1	32	0	0	0	38	0	0	0
N	+ Adh-E + / + Adh-E +	Lp Adh-C ae / + Adh-C ae	1	18	0	0	0	17	0	0	0
O	Lp Adh-C ae / + Adh-E +	+ Adh-C ae / + Adh-C ae	3	87	0	0	0	0	0	0	68
P	+ Adh-E + / Lp Adh-C ae	+ Adh-C ae / + Adh-C ae	3	70	0	0	0	0	0	0	54
Q	+ Adh-C ae / + Adh-C ae	Lp Adh-C ae / + Adh-E +	7	76	10	34	24	35	36	0	64
R	+ Adh-C ae / + Adh-C ae	+ Adh-E + / Lp Adh-C ae	9	129	10	66	47	40	60	4	120

* f indicates number of families tested.

of the extra long palpi females died during development. Of the total 3242 eggs laid by the above 20 mothers, 2755 produced viable larvae (85%) and 67% of these larvae developed to the adult stage. The 70 females with extra long palpi were released into a cage with twice that number of males. Mice and oviposition cups were offered daily after the third and sixth day respectively for 30 days. Al-

Table 3. Chi square analysis of cross results.

Cross	Chi square testing for 1:1 segregation ♀:♂	+:Lp[1]	+:bw	+:ae	CE:C	Chi squares testing independence[1] Lp-Adh	Lp-bw	Lp-ae	bw-ae	Adh-ae
A	0.28	0.38	–	–	–	–	–	–	–	–
B	0.26	1.06	–	–	–	–	–	–	–	–
C	0.04	1.13	–	–	–	–	–	–	–	–
D	0.97	0.80	–	–	–	–	–	–	–	–
E	0.30	0.30	0.30	0.30	–	–	161**	161**	161**	–
F	0.10	0.21	1.86	1.86	–	–	121**	121**	121**	–
G	2.86	1.49	1.03	1.03	–	–	172**	172**	172**	–
H	2.20	2.17	1.01	1.01	–	–	203**	203**	203**	–
I	0.18	0.19	4.69*	0.42	–	–	25.2**	5.82*	418**	–
J	5.75*	5.39*	4.66*	6.54*	–	–	14.7**	3.57	227**	–
K	0.33	0.14	28.6**	18.2**	–	–	34.4**	2.11	177**	–
L	0	1.51	6.03**	5.31*	–	–	16.0**	11.3**	197**	–
M	–	0.51	–	–	–	–	–	–	–	–
N	–	0.03	–	–	–	–	–	–	–	–
O	–	2.33	–	2.33	2.33	155**	–	155**	–	155**
P	–	2.06	–	2.06	2.06	124**	–	124**	–	124**
Q	–	0.29	–	4.39*	0.43	71.2**	–	1.58	–	50.8**
R	–	1.65	–	0.01	0.01	158**	–	5.25*	–	80.7**

[1] = female progeny only.

* $P < 0.05$.

** $P < 0.01$.

Table 4. Frequency of recombination among tested loci.

Cross	Lp-Adh	Lp-bw	Lp-ae	bw-ae	Adh-ae
I	–	40.06±1.93	49.61±1.97	9.55±1.16	–
J	–	39.65±2.64	48.98±2.69	9.33±1.57	–
K	–	34.20±2.55	48.40±2.69	14.20±1.89	–
L	–	39.32±2.60	51.86±2.66	12.54±1.76	–
Q	24.73±2.58	–	53.40±2.98	–	28.67±2.70
R	21.22±1.87	–	50.63±2.29	–	29.41±2.08
Total Data	22.52±1.52	38.62±1.18	50.31±1.01	11.08±0.76	29.14±1.65

though the females took a blood meal, no fertile egg rafts were laid. At the end of 30 days, 44 females still alive were dissected and the ovaries and spermathecae were examined for development and sperm respectively. Only 4 females were fertilized, with 1 appearing gravid. Of the 40 unfertilized females, 16 had developed eggs. Thus, it appears that these presumptive homozygous *Lp* females do not readily mate or lay eggs. It is not surprising, therefore, that a true-breeding *Lp* strain has not been successfully isolated.

In order to test for the occurrence of possible homozygous *Lp* males, all the resulting wild type males from 3 of the 20 families

$$\left(\frac{Lp\ +\ +}{+\ +\ +}\ \text{♀}\ \times\ \frac{Lp\ +\ +}{+\ bw\ ae}\ \text{♂}\right)$$

were individually test mated to wild type virgin females. Of the 46 males which produced progeny, 8 had all + daughters, 28 had both + and *Lp* daughters and 10 had all *Lp* daughters. Thus, it would appear that *Lp*/*Lp* males are viable and fertile and are produced in approximately the expected proportion in a heterozygous mating.

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