GENETIC ANALYSES OF HEAT-SENSITVE PAWN MUTANTS OF *PARAMECIUM AURELIA*[1]

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ABSTRACT

Heat-sensitive Pawn (*ts* Pawns) of *Paramecium aurelia* behaved normally when grown at 23° but failed to avoid strong stimuli at 35°. Four of the five *ts* Pawn lines tested were found to be allelic at a locus known also to carry temperature-independent Pawn mutations. The fifth *ts* Pawn line complemented all the conditional and unconditional Pawn mutants of the two known loci. This result, together with the patterns of F_2 segregation from various crosses, suggested the existence of a third Pawn locus. An additive effect of the unlinked *ts* Pawn genes was observed. These findings and the significance of *ts* Pawns as experimental material in behavioral and physiological research are discussed.

BEHAVIORAL genetics of *Paramecium aurelia* has been developed aiming at a genetic dissection of the excitable membrane. Mutants with altered membrane properties resulting in insensitivity or over-reactivity to cationic stimuli have been described (KUNG 1971a,b). We report here results on the genetic analyses of conditional behavioral mutants discovered recently.

The locomotor behavior of Paramecium is under the control of its surface membrane. The beating direction and frequency of the cilia are correlated with the cross membrane potential (ECKERT 1972; ECKERT and NAITOH 1972). Specifically, reversal of the ciliary power strokes is caused by membrane depolarization. The depolarization resulting from suprathreshold stimuli triggers active electrogenesis leading to the generation of action potentials. This process is analogous to Na-activation in most nerve and muscle, although in the case of Paramecium membrane Ca^{++} instead of Na^{+} appears to carry the action current. The resultant increase of internal Ca^{++} concentration activates the reversal of the ciliary beating direction. The behavioral correlate of ciliary reversal is a period of backward movement, a major component in the "avoiding reactions" used by the cell in reaction to various stimuli (JENNINGS 1906).

One group of the behavioral mutants reported by KUNG (1971a) is unable to swim backward in the face of a stimulus and was therefore named "Pawn". The loss of avoidance appears to be general since neither mechanical stimulation at the anterior end nor various kinds of cation succeed in generating the avoiding

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reaction. Electrophysiological studies through intracellular recording revealed the loss of active electrogenesis in these mutants, although their passive membrane properties appeared unchanged (KUNG and ECKERT 1972; SATOW and KUNG 1973). The Pawn gene mutation appears to lead to a functional defect in the mechanism responsible for the voltage-sensitive Ca^{++} conductance changes on the membrane. KUNG and NAITOH (1973) showed that models of Pawns whose membrane was disrupted by extraction of Triton-X 100, could swim backward when enough Ca^{++} and adenosine triphosphate were added to the medium. Thus, the lack of backing ability in Pawns is caused purely by the membrane defect; i.e., the Pawn mutation has no pleiotrophic effect on the ciliary motile apparatus.

The Pawn phenotype as an indicator of failure of active electrogenesis is very important in our quest for the identity of the molecular mechanism in membrane excitation. Although only two unlinked loci which give this phenotype have been identified (KUNG 1971b), over one hundred lines of Pawns are now available. This makes possible an exhaustive search, now in progress, for the possible genic loci leading to this peculiar trait.

We have recently extended our study to conditional behavioral mutants. Heat-sensitive mutants have been successfully exploited in the studies of different biological systems, from the morphogenesis of phage particles (WOOD *et al.* 1968) to the nervous system of flies (SUZUKI, GRIGLIATTI and WILLIAMSON 1971). Temperature-sensitive behavioral mutants in Paramecium may help us gain insight into the nature of the mutated membrane element since they should harbor mutated macromolecules whose functional configuration can be disrupted and restored by simple changes of experimental temperature.

MATERIALS AND METHODS

P. aurelia of syngen 4 were used throughout. The stocks used were: 51s (non-kappa bearing); d4–93 (previously known as body deformation mutant, genotype *bd bd*); d4–95 (previously Pawn 1–2–34, genotype *pwB pwB*); d4–94 (previously Pawn 5–5–28, genotype *pwA pwA*) and five lines of heat-sensitive Pawns (*ts* Pawns) recently discovered. The characteristics and origin of these strains are summarized in Table 1. The behaviorally-normal, bodily-deformed mutant, d4–93, was used throughout this study to provide a genetic marker. Clones of d4–93 had cells of various degrees of morphological abnormality from slightly twisted bodies to monstrous distortions. The body deformations did not interfere with the behavioral diagnosis. The five *ts* Pawn strains are given the code letters A to E in this paper, for ease in reading. Four lines are given the standard d4 designations for derived stocks of syngen 4 *P. aurelia*. The fifth is not so assigned because it is most likely to be identical to line D (d4–134). Information on the origin of the strains given in Table 1 is important in the understanding of the genic relation of various Pawns (see below).

Cells were grown in Cerophyl medium bacterized with *Aerobacter aerogenes* (SONNEBORN 1970) either at room temperature, 23 ± 1°, or in an incubator at 35 ± 1°. Methods of mutagenesis and screening for heat-sensitive Pawns were those reported by CHANG and KUNG (1973). The diagnostic characteristic of these Pawns was that they could perform the avoiding reactions when they were grown at room temperature but not at 35°. This contrasted with the wild-type strains, which were able to avoid, and the unconditional Pawns which were not, regardless of the temperature (CHANG and KUNG 1973). The ability to avoid was tested by collecting 10 to 30 animals from the culture medium with a micropipette and transferring them under a

TABLE 1

Characteristics of strains of P. aurelia

Strain	Code	Present of avoiding reaction* 23°†	35°	Origin
Behaviorally normal strains				
51s		yes	yes	derived from natural stock (see SONNEBORN 1959)
d4–93		yes	yes	mutagenized September 1968 (KUNG 1971b)
Unconditional pawns				
d4–94		no‖	no	mutagenized October 1969 (KUNG 1971b)
d4–95		no	no	mutagenized October 1969 (KUNG 1971b)
Temperature-sensitive pawns				
d4–131	A	yes	no	mutagenized August 1972‡
d4–132	B	yes¶	no	mutagenized August 1972‡
d4–133	C	yes	no	mutagenized July 1972
d4–134	D	yes	no	mutagenized August 1972§
pw 8–2–39	E	yes	no	mutagenized August 1972§

* Tested with a Ba++ solution. "Yes" indicates the presence of violent avoidance through backward swimming. "No" indicates a complete absence of avoiding reaction. See text for description.

† Temperatures in which the cells were grown and tested.

‡ These two lines came from the same mutagenized exautogamous population.

§ These two lines came from the same mutagenized exautogamous population.

‖ Weak avoiding reactions were sometimes observed at the wall of the culture vessels but no avoidance to Ba++ was seen.

¶ The backward movement of this line upon Ba++ stimulation is slower than that of the wild type.

dissecting microscope into a depression containing a test solution of 24 mM $BaCl_2$, 1 mM $CaCl_2$, 1 mM Tris, pH 7.2. This was a toxic solution, to which the normal cells reacted with violent avoiding reactions—i.e., rapid backward movement along tight righthanded helical courses. Reactions to this Ba-solution provided a stringent test for the ability to avoid. Cells exhibiting the typical Pawn phenotype swam forward in loose left-handed helices, the paths paramecia usually swim. We found that phenotypic transition occurred five hours after the *ts* Pawns tested were put in the restrictive temperature and that the transition was complete within seven hours. To ensure correct phenotyping of the F_1 and F_2, well-fed clones were replicated and grown at 35° for more than twelve hours before testing. The original sets of clones were used for phenotypic studies at 23°, while the replicated sets were studied at 35°.

Movement of the cells could be registered with a long-exposure dark-field photomacrographic method (DRYL 1955; KUNG 1971a). A convenient Polaroid version of the photographic method (CHANG and KUNG 1973) was used in this study. Throughout this study, the F_1 was derived from conjugations of parents and the F_2 from autogamy of F_1. In autogamy, the two identical haploid gametic nuclei, derived from the same meitotic product, fuse to restore diploidy. The process results in homozygosity at all loci. For detailed descriptions of conjugation and autogamy and techniques of genetic manipulation, see BEALE (1954) and SONNEBORN (1970).

RESULTS

Pattern of Inheritance of the ts-*Pawn Trait*

The phenotype of a variant clone of ciliates can be the result of mutation in the micronuclei, macronuclei, replicating cytoplasmic particles or cortical structures.

TABLE 2

Crosses between ts-Pawns and d4–93

Cross	F_1	F_2 AR* at 23° No : Yes	F_2 AR at 35° No : Yes	Marker BD† : wild type
‡A × d4–93	wild type	0 : 117	58 : 59	61 : 56
B × d4–93	wild type	0 : 115	60 : 55	58 : 57
C × d4–93	wild type	0 : 119	52 : 67	59 : 60
D × d4–93	wild type	0 : 117	57 : 60	65 : 52
E × d4–93	wild type	0 : 58	26 : 32	27 : 31

* AR stands for avoiding reactions. "Yes" and "No" represent, respectively, the presence and absence of avoidance to the test solution.
† BD stands for body deformation, the trait of the d4–93 parent and all *bd bd* homozygotes.
‡ The codes in this column refer to the *ts*-Pawn lines. See Table 1.

It is therefore important to observe first the pattern of inheritance of the phenotype with which we are concerned.

All *ts*-Pawn lines were crossed to d4–93 and the phenotypes of F_1 and autogamous F_2 were scored. The recessive gene *bd* in stock d4–93 served as a genetic marker to check the validity of the crosses. The results of these crosses are summarized in Table 2.

All F_1 were wild type in body shape. The disappearance of the d4-93 character in F_1 and the segregation of it into half of the autogamous F_2 showed that we had true crosses through conjugations of the parents in each case. When F_1 were tested for their behavior, they all performed avoiding reactions at 23° and 35°. The F_2 grown and tested at 23° all avoided the test solution. However, about half of the F_2 in each case failed to show any avoidance when grown and tested at 35°. The simplest explanation consistent with this set of data is that in each strain the *ts-Pawn* phenotype is caused by a pair of recessive alleles.

No linkage was observed between the marker gene and genes for *ts*-Pawn traits.

Genic Relation of the Five ts-Pawn Strains

After establishing the truly nuclear genic nature of the *ts*-Pawn trait, we proceeded to analyze the relation of the genes involved testing for complementation, linkage, allelism and genic additive effect. The original *ts*-Pawn lines were crossed to various *ts*-Pawn clones selected from the crosses in the previous section furnishing the proper mating types and genetic marker. The results (Table 3) fall into two patterns.

1) All crosses *not* involving line A fell into a simple pattern. The F_1 were all *ts*-Pawns, and failed to avoid at the restrictive temperature. Although the marker segregated normally, there was no segregation of the *ts*-Pawn phenotype among the F_2. All the F_2 could avoid at 23° but not at 35°. Thus, all the alleles responsible for the *ts*-Pawn phenotype in lines B, C, D and E appeared to be at the same locus. Line C was isolated in a separate mutagenic experiment from the other lines and is definitely the result of a different mutation (Table 1). Lines B and

TABLE 3

Crosses between ts *Pawns*

Cross	F_1		F_2 AR at 23°		F_2 AR at 35°		Marker BD : wild type
	AR at 23°	At 35°	No	Yes	No	Yes	
A × B	Yes	Yes	63	172	166	69	121 : 114
A × C	Yes	Yes	27	75	70	32	46 : 56
A × D	Yes	Yes	33	86	85	34	60 : 59
A × E	Yes	Yes	31	87	82	36	68 : 50
B × C	Yes	No	0	95	95	0	51 : 44
B × D	Yes	No	0	120	120	0	— —*
B × E	Yes	No	0	72	72	0	37 : 35
C × D	Yes	No	0	120	120	0	56 : 64
C × E	Yes	No	0	108	108	0	48 : 60
D × E	Yes	No	0	99	99	0	48 : 51

* Marker was not used in this cross. Cytological examination showed 100% autogamy of F_1, as in the other crosses, when F_2 were isolated.

D were derived from two of the sixteen fractions separated immediately after mutagenesis and are thus very likely the results of two separate mutagenic hits. The fact that line B had slower backward movement upon Ba++ stimulation also suggests that they are from different mutations. Lines D and E, however, were derived from the same fraction of one mutagenic experiment and have identical phenotypes. That these two mutant lines were found to be isogenic is a further indication of their common origin. Since D and E may be sister lines descended from the same mutant, it is safer not to consider one of them a separate derived stock. This is why line E was not given the standard d4 designation (Table 1).

2) All crosses involving line A as one parent fell into a different pattern. The F_1 of such crosses were wild type. When the F_2 were tested at 23°, roughly one-fourth had no avoiding reactions, but two-thirds of those which avoided failed to do so when later tested at 35°. In other words, at the restrictive temperature three-fourth instead one-fourth of the F_2 failed to avoid. The phenotypic ratio of the F_2 was therefore: Pawn : *ts* Pawn : wild type = 1 : 2 : 1.

The actual ratios of the four crosses derived from the data in Table 3 are:

63 : 103 : 69 for the F_2 of cross A × B,
27 : 43 : 32 for the F_2 of cross A × C,
33 : 52 : 34 for the F_2 of cross A × D,
31 : 51 : 36 for the F_2 of cross A × E.

Although the second term (*ts* Pawn) was consistently short of half of the total, each set of results is not significantly different from the 1 : 2 : 1 expectation. The marker segregated normally in the F_2 of all crosses, showing that all the crosses were valid.

The simplest explanation seems to be the following. All the genes involved in giving the *ts-Pawn* phenotype are recessive. The mutation in line A is completely

unlinked to the one in the other lines. Double mutants carrying two sets of such mutations are unconditional Pawns, lacking the avoiding reaction even in the permissive temperature. Thus, after F_1 autogamy, the F_2 consists of half parental type (*ts* Pawn) and half recombinant types including one-fourth wild-type recombinants and one-fourth double mutants (unconditional Pawns).

It was not expected that the two unlinked conditional Pawn mutations would lead to an unconditional phenotype. Although the numerical results of these crosses were consistent with this hypothesis, we further tested the presumed double homozygous lines by crossing them with behaviorally-normal strains.

An F_2 line, r40, was chosen from cross A × B. This line behaved as an unconditional Pawn and also carried the morphological trait of the marker gene. When r40 was crossed to 51s, the F_1 were wild type and the autogamous F_2 had the phenotypic ratio:

unconditional Pawn : *ts* Pawn : wild type = 29 : 55 : 29
(Marker segregated as body deformation (BD) : wild type = 63 : 50).

Another line, r7, also from cross A × B, was crossed to the behaviorally-normal, marker-carrying strain d4-93. The F_1 were wild type and the autogamous F_2 had the ratio:

unconditional Pawn : *ts* Pawn : wild type = 26 : 41 : 29
(Marker segregated as BD : wild type = 48 : 48).

While both sets of marker segregation satisfy the χ^2-test for 1 : 1 ratio, both sets of data on the segregation of behavioral traits are consistent with the 1 : 2 : 1 expectation. Thus, there is little doubt that the unconditional Pawns resulting from the crosses of two unconditional Pawn parents are double homozygotes carrying both sets of parental mutations.

The distinction between the parental lines and the double mutant filial lines of the A × B cross can be seen easily in Figure 1. This figure shows the reaction of paramecia to a Ba-solution containing 4 mM $BaCl_2$, 1 mM $CaCl_2$, 1 mM Tris, pH 7.2. In Figures 1A and 1B, line A and line B were tested, respectively. Since the test was performed at 23°, a permissive temperature, both of these *ts*-Pawn lines performed the repeated avoiding reactions which caused the cells to jerk back and forth about the spot in which they were put. Figure 1C shows the reaction to this Ba-solution of line r7, the double mutant harboring both the A and B genic mutations. The cells swam away in a sunray pattern into the surrounding Ba-solution, which they obviously did not avoid. A detailed description of the behavior of the conditional Pawns is given in CHANG and KUNG (1973).

Genic Relation of the ts *Pawns and the Unconditional Pawns*

Mutants that showed the Pawn phenotype at all temperatures carried mutations in one of the two possible loci (KUNG 1971b). We proceeded to cross these Pawns to the *ts* Pawns to see how they were related.

The result of the crosses between the *ts*-Pawn lines and Pawn d4-95 is summarized in Table 4. The F_1 had avoiding reactions at both 23° and 35°, like the

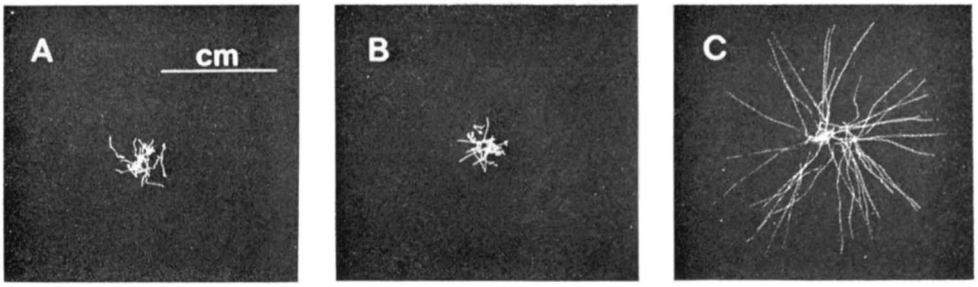

FIGURE 1.—Comparison of the reactions to Ba++ by two single *ts*-Pawn mutant strains and the double mutant derived from them. Cells were collected in a drop of culture fluid and added perpendicularly into the center of a thin pool of Ba-solution (4 mM $BaCl_2$, 1 mM $CaCl_2$, 1 mM Tris pH 7.2) just before the camera shutter opened for 13.3 ± 0.1 sec. The dark field is the pool of solution and each white line represents the trajectory traversed by a paramecium during the time of film exposure. For a detailed description of the techniques of dark-field Polaroid photomacrography see CHANG and KUNG (1973). A and B show the reaction to Ba-solution by the cells of lines A and B, respectively. The repeated avoiding reactions confined the cells near the spot in which they were placed. In C, cells of line r7, a double mutant derived from the cross between A and B, did not avoid Ba++. They simply swam away from the drop along their usual helical paths.

wild type. When the autogamous F_2 clones were tested for their ability to avoid the test solution, roughly half of them could avoid if tested at 23°. However, among those F_2 clones that could avoid, about half of them failed to do so when tested at 35°. In other words, the ratio of clones with avoiding reaction to those without changed from 1 : 1 to 1 : 3 when the temperature was raised. This means the phenotypic ratio of the F_2 was: unconditional Pawn : *ts* Pawn : wild type = 2 : 1 : 1. The observed ratios of autogamous F_2 segregation of the five crosses involving line A to line E were as follows:

58 : 27 : 34 in cross A × d4-95
53 : 28 : 39 in cross B × d4-95

TABLE 4

Crosses between ts *Pawns and Pawn d4–95*

Cross	F_1		F_2		Marker
	AR at 23°	At 35°	AR at 23° No : Yes	AR at 35° No : Yes	BD : wild type
A × d4–95	Yes	Yes	58 : 61	85 : 34	— —*
B × d4–95	Yes	Yes	53 : 67	81 : 39	— —
C × d4–95 (a)	Yes	Yes	56 : 57	96 : 17†	— —
(b)	Yes	Yes	56 : 57	86 : 27	— —
(c)	Yes	Yes	54 : 61	82 : 33	52 : 53
D × d4–95	Yes	Yes	63 : 52	84 : 31	— —
E × d4–95	Yes	Yes	66 : 50	95 : 21	— —

* — means that the marker was not available for the cross at the time.
† This ratio is statistically different from the 3 : 1 expectation.

166 : 98 : 77 in cross C × d4-95
63 : 21 : 31 in cross D × d4-95
66 : 29 : 21 in cross E × d4-95.

None of these ratios is significantly different from the 2 : 1 : 1 expectation.

These data indicate that the Pawn gene for d4-95 (*pwB*) is completely unlinked to either the gene for line A or the one for the rest of the *ts*-Pawn lines. *pwB* is apparently epistatic over the *ts*-Pawn genes.

When the *ts*-Pawn lines were crossed to Pawn d4-94, some very interesting results were obtained. They are given in Table 5. Lines B, C and D did not complement d4-94. The F_1 of the crosses between these lines and d4-94 were leaky Pawns in phenotype. They had a greater tendency to show weak avoiding reactions when the cells came to the edge of the depression than the d4-94 parents had. The F_1 of cross C × d4-94 behaved even less like Pawns. Unlike the F_1 of the other two crosses, weak backward movement for short distances was observed in some of the F_1 cells of this cross when tested with the Ba-solution.

Half of the autogamous F_2 clones of these crosses avoided the Ba-solution at 23°, but none did at 35°. This means that half of the F_2 are *ts* Pawns, like one of the parents, and half are unconditional Pawns, like the other parent. This result implies that the gene for the *ts*-Pawn phenotype in lines B, C and D is allelic to *pwA*, which is responsible for the unconditional Pawn phenotype in d4-94.

The F_1 of the cross A × d4-94 were wild type. Among the autogamous F_2, 98 clones gave violent avoiding reactions to the Ba++ test at 23°. When tested at 35°, 12 of these clones lost the ability to avoid. Thus the phenotypic ratio of F_2 is:

unconditional Pawns : *ts* Pawns : wild type = 79 : 12 : 86.

Since there is complementation between line A and d4-94 and there is segregation of the phenotypes in the F_2, the two mutations involved are not allelic. The significant difference from the 2 : 1 : 1 expectation is discussed below.

CONCLUSIONS AND DISCUSSION

This study suggests the presence of two genetic loci that can produce the temperature-sensitive-Pawn phenotype.

TABLE 5

Crosses between ts *Pawn and Pawn d4–94**

Cross	F_1		F_2		Marker
	AR at 23°	At 35°	AR at 23° No : Yes	AR at 35° No : Yes	BD : wild type
*A × d4–94	Yes	Yes	79 : 98	91 : 86	85 : 92
B × d4–94	No	No	48 : 43	91 : 0	41 : 54
C × d4–94	No	No	51 : 65	116 : 0	59 : 57
D × d4–94	No	No	63 : 54	117 : 0	56 : 51

* d4–94 and some of the descendants of the crosses here did not avoid the Ba++ test solution but sometimes gave very weak avoiding reactions to the wall of the culture vessels.

Since lines B, C and D, as well as stock d4-94 (genotype *pwA pwA*), were found to be of the same complementation group (see Table 5), the genes for lines B, C and D (now stock d4-132, d4-133 and d4-134) are now designated as pwA^1, pwA^2, pwA^3 respectively, all allelic to *pwA*. However, cells of *pwA pwA* genotype (d4-94) are unconditional Pawns. In other words, mutations were induced in the *pwA* locus many times, resulting in both temperature-independent and temperature-sensitive phenotypes. Although this is not unexpected, it appears to be the first time such a phenomenon has been recorded in the genetics of ciliates.

Since the heterozygotes pwA^1/pwA, pwA^2/pwA and pwA^3/pwA are leaky Pawns, having a phenotype between the *ts*-Pawn parents and the d4-94 parent, no clear dominance relation can be asserted.

The evidence indicates that line A (now stock d4-131) is mutated at a gene, now designated as *pwC*, not previously identified. This is deduced from the following findings:

a) Line A complements d4-95 (genotype *pwB pwB*) and its mutation is completely unlinked to *pwB* (see Table 4).

b) Line A complements lines B, C and D (genotype pwA^1pwA^1, pwA^2pwA^2, and pwA^3pwA^3, respectively) and its mutation is completely unlinked to pwA^1, pwA^2 and pwA^3 (see Table 3). Since pwA^1, pwA^2 and pwA^3 are allelic to *pwA*, the gene of line A in question is unlinked to *pwA*. This conclusion is reinforced by the presence of phenotypically and genotypically identifiable double mutants such as line r7.

c) Line A complements d4-94 (genotype *pwA pwA*) (see F_1 of Table 5) and there is segregation of the three relevant phenotypes in the F_2 of the A × d4-94 cross (see F_2 of Table 5).

The pattern of F_2 segregation of the cross A × d4-94 does not fit the expectation of a simple dihydrid cross with the unconditional phenotype epistatic over the conditional one. Bias due to technical difficulties, such as mixture of F_1 in F_2 from incomplete autogamy or macronuclear regeneration, is not likely because the *bd* marker segregated normally in this cross. It is conceivable that other genic components may be involved. Such components could have a modifying function which causes the *pwA pwA* homozygote to be slightly leaky, as we have observed (see note on Table 1), and would cause the *pwC pwC* homozygote to become indistinguishable from the wild type at 35° under our criterion of the Ba^{++} test. If, for instance, two such modifiers exist in d4-94 and if they are unlinked to the genes in question, the expectation of F_2 segregation of Pawn : *ts* Pawn : wild type in the cross line A × d4-94 should be 8 : 1 : 7, with which the result obtained is compatible. Future analyses classifying the leaky Pawns intermediate between the typical Pawn and typical wild type using other test solutions may clarify this situation.

It is interesting that although d4-131 (genotypes *pwC pwC*) and d4-132 (pwA^1 pwA^1) are both heat-sensitive Pawns, the double mutants (*pwC pwC*, pwA^1 pwA^1) are Pawn but temperature-independent. This additive effect of the two unlinked genes suggests that the two gene products do not function normally

even at the permissive temperature, although each one of them alone has no observable expression at lower temperature.

These temperature-sensitive mutants indicate the presence of heat disruptable macromolecular configurations important in the Ca-activation-related membrane structure. However, the gene products may not necessarily be the structural components of the membrane, although they must certainly affect these components. Active growth and synthesis are apparently needed for the phenotypic changes (CHANG and KUNG, in preparation). Thus the *ts*-Pawn gene product may function in the synthesis and organization of the relevant membrane structure (such as the voltage-sensitive Ca-gate) and may not be one of the structural components. The finding that there are at least three loci, the mutations of which lead to the Pawn phenotype, reinforces the view that the correct function of many macromolecules is needed to ensure normal membrane excitation.

Our success in finding these *ts* Pawns opens a new area in which we can select mutants carrying various defects of the membrane processes. Even vital membrane functions can now be tackled with the search for heat-sensitive mutants during temperature transition or at moderately high temperatures. Conditional mutants allow us to turn on and off specific membrane processes at will. This, therefore, provides us an easy handle in comparing the normal and altered structures and functions. The relationship of the Pawn genes elucidated in this paper will be useful in the studies of the Pawn gene products whose wild-type counterparts are apparently required for proper membrane excitation.

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