Perca flavescens Mitchill. J. Fish. Res. Board Can. 34:1504-1514.

Timmons, T. J., W. L. Shelton, W. D. Davies. 1980. Differential growth of largemouth bass in West Point Reservoir, Alabama-Georgia. Trans. Amer. Fish. Soc. 109:176-186.

Timmons, T. J., W. L. Shelton, and W. D. Davies. 1981. Food of largemouth bass during the first three years after impoundment of West Point Reservoir, Alabama-Georgia. J. Tennessee Acad. Sci. 56:23-27.

Wells, L. R. 1980. Food of alewives, yellow perch, spottail shiners, trout perch, and slimy and fourhorn sculpins in Southeastern Lake Michigan. U. S. Fish Wildl. Serv. Tech. Pap. 98:12 p.

NEW DISTRIBUTION RECORD FOR THE BARKING TREEFROG, *HYLA GRATIOSA* LeCONTE, IN WESTERN TENNESSEE

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BRIEF REPORT

On 23 June 1983, after a brief thunderstorm, a barking treefrog *(Hyla gratiosa)* was found on the patio of a home in suburban Barlett, Shelby County, Tennessee. This constitutes the first record of this species in Shelby County and the second locality in the Coastal Plain of west Tennessee (Eagar and Hatcher, 1980; Jacob, 1980).

The barking treefrog is confined mostly to the Coastal Plain from southeastern Virginia south to southern Florida and west to eastern Louisiana. It is also recorded from isolated populations from upland physiographic provinces in northern Alabama and northern Georgia and has been successfully introduced into southern New Jersey (Conant, 1980; Caldwell, 1982). It is reported from three southwestern counties in Kentucky, apparently its northernmost point of distribution in the central United States (Harker, et al., 1980). Outside Shelby County *Hyla gratiosa* is known from Franklin, Montgomery and White Counties

in central Tennessee (Eagar and Hatcher, 1980) and Hardeman County in western Tennessee (Jacob, 1980).

This species occupies a variety of habitats, but in Tennessee has been found usually associated with isolated ponds (Eagar and Hatcher, 1980). The nearest permanent body of water to this sighting is a man-made pond of about 1.5 hectares, located approximately 100 meters away.

LITERATURE CITED

Caldwell, J. P. 1982. *Hyla gratiosa*. Cat. Amer. Amphib. Reptiles. pp 298.1,298.2.

Conant, R. 1980. A field guide to reptiles and amphibians of eastern central North America. Houghton Mifflin Company, Boston. 429 pp.

Eagar, D. C. and R. M. Hatcher, Editors.1980. Tennessee wildlife. Volume 1: The vertebrates. Tennessee Wildlife Resources Agency, Department of Conservation, Nashville. 414 pp.

Harker, D. F. Jr., M. E. Medley, W. C. Houtcooper, and A. Phillippi. 1980. Kentucky natural areas plan. Appendix A. Kentucky Nature Preserves Commission, Frankfort.

Jacob, J. S. 1980. Geographic distribution: *Hyla gratiosa*. SSAR Herpetol. Rev. 11:36-37.

LEG AND WING PHENOTYPES RESULTING FROM THE INTERACTION OF THE MUTANTS CUBITUS INTERRUPTUS-WALLACE (ci^w) AND − DOMINANT (ci^D) IN DROSOPHILA MELANOGASTER

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ABSTRACT

The dominant ci^W and ci^D mutants produce wing vein interruptions and a widening of the wing blade. In addition, ci^W has an effect on the legs. Although the ci^W/ci^D genotype is an early developmental stage lethal, twelve individuals which survived and completed their development have been recovered and are described. The proximal wing regions and the L3 wing vein are unaffected, but there are interruptions and widening of other wing veins. The legs have extra bristles, growths, incomplete joints, increased diameter, and fusions of the femur and tibia. The presence of extra structural elements and increased diameter suggests increased mitotic activity, but the failure of complete joint formation suggests a loss of positional reference. Both of these effects appear to be localized suggesting an influence on specific cell clones.

INTRODUCTION

The fourth chromosome cubitus interruptus mutants are named for their effect on the fourth longitudinal, cubital, wing vein. Both cubitus interruptus—Wallace (ci^W; 4-0.0) and cubitus interruptus—Dominant (ci^D; 4-0.0) are dominant and have pleiotropic effects. ci^D produces a widening and warping of the wing blade. ci^W produces a broadening of the wing, elimination of veins in the most extreme expression, and effects eyes, antennae and legs (see Lindsley and Grell, 1968, for complete description). ci^D is homozygous lethal. ci^W shows a dosage effect in that ci^W/deficiency flies have wings which are more nearly normal but legs which are more extremely affected (Stern and Schaeffer, 1943).

Evidence has been presented that these two mutants are not alleles, and ci^D has been placed to the right of ci^W on the fourth chromosome (Hochman, 1973; Lindsley and Grell, 1968). In addition, Hochman showed that the ci^W/ci^D heterozygote is lethal with most individuals dying in an early stage of development. The report which follows is a description of the phenotype observed in ci^W/ci^D heterozygotes which survived and became fully developed adults.

METHODS AND MATERIALS

Heterozygous ci^W/ci^D flies were produced by crossing ci^D/ey^D (eyeless-dominant; 4-2.0) flies to homozygous ci^W individuals. The flies which eclosed and could be removed from the culture containers were ci^W/ey^D. A small number of individuals were removed from the media in which they had become stuck. These flies were ey^+, indicating that they carried the ci^D mutant. Unopened pupal cases also proved to be ey^+, ci^W/ci^D flies. All cultures were reared on a corn meal—yeast—molasses—agar media at $25 \pm 1°C$.

A total of 12 ci^W/ci^D flies were recovered. The legs and the wings were removed and mounted in euparal for examination under a compound microscope. Most of the wings had not been inflated, even on enclosed flies, and had to be teased out into a normal flat blade configuration. Measurements were made using an occular micrometer. Drawings were made with a camera lucida.

RESULTS

Ten female and two male flies were recovered. Eighteen wings were removed, but four of them could not be spread and flattened sufficiently to allow an accurate determination of the condition of all their features. Ten of the wings were broader than normal (Figure 1A), a characteristic of both ci^W and ci^D. This was most extreme on the distal portion of the blade. One wing appeared to be narrower than normal.

The wing vein pattern was modified on fourteen wings. The third longitudinal vein was present and complete on all of these wings. The fourth longitudinal vein was absent from the twelve wings where a determination could be made. On four wings a short spur persisted at the cross vein. Eight of the wings had the fifth longitudinal veins interrupted. Only one wing had a complete fifth vein. The remainder were unclear. The missing portions ranged from short distal regions to almost total absence of a distal or proximal region. All fourteen of these wings had a second longitudinal vein which failed to reach the wing margin and terminated in a forked structure (Figure 1). In

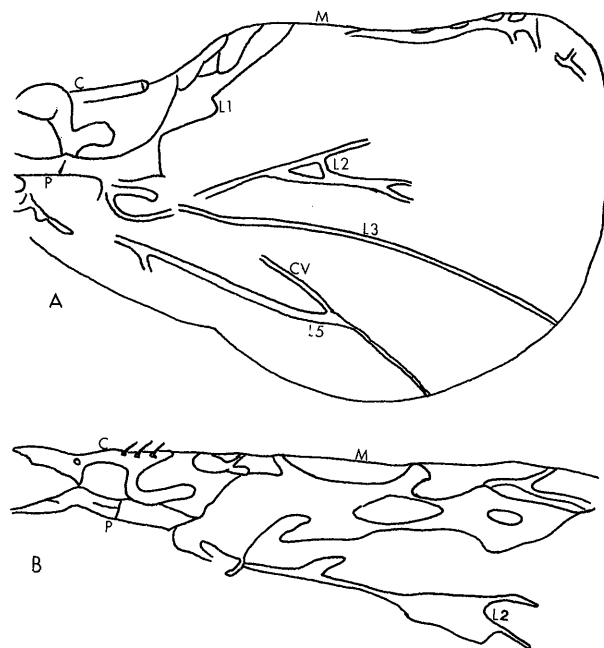some cases there were small corssveins running between parallel longitudinal segments.

FIG. 1. *Venation pattern typical of the wings ci^W/ci^D flies. A. Wing Blade. B. Enlargement of the proximal anterior wing margin. (L1-L5) Longitudinal veins 1-5; (M) Marginal vein; (C) Costal vein; (P) Proximal radial vein; (CV) Posterior crossvein.*

Three wings had enlarged first longitudinal and marginal veins (Figure 1A, 1B). The proximal anterior region of the wing was normally lined with short heavy bristles termed the triple row bristles. On these three wings, and two others (both from the same fly) which had normal appearing marginal veins, the triple row bristles were absent. The proximal hinge structure, the costal veins and the remaining wing margins were normal.

All but two of the 62 legs recovered from these flies had elevated bristle numbers, growths in or on the cuticle, fusion of the tibia and femur along a portion of their lengths, and incomplete joint formation between tarsal segments (Figure 2). A single fly always had more than one leg affected and many legs had more than one aberration. All segments from the femur to the fifth tarsus were affected.

Table 1 lists the numbers of selected bristles observed on legs. Figure 2B shows the sexcomb arrangement on one of the male first leg basitarsus. The extra bristles occurred singly or in clusters of two to five. There were thirty-one sexcomb bristles and four heavy pointed bristles lying immediately adjacent to the sexcomb. The sexcomb bristles were arranged in an oblique row, with two ventrally located bristles instead of the normal one. Figure 2C shows a distal tibia with two preapical and two apical bristles. The transverse row bristles are found on the distal end of

FIG. 2. *Aberrations present on the legs of ci^W/ci^D flies. A. Complete leg with femur-tibia fusion, enlarged tarsal segments and an incomplete joint between segments T4 and T5. B. Male basitarsus showing the increased number and the disarray of the sexcomb bristles. C. Distal region of a second leg tibia with duplication of preapical and apical bristles. D. Distal region of the tibia of a first legs showing the irregular transverse row bristle patterns. E. Leg with incomplete joint between T4 and T5, a growth within T5 and duplication of one claw. F. External growth on T3. T2 is enlarged. (FE) Femur; (TI) Tibia; (B) Basitarsus; (T2-T5) Tarsal segments 2-5; (A) Apical bristle; (PA) Preapical bristle.*

the tarsus and the ventral surface of the basitarsus (Hanna-Alava, 1958). Normally, there were six orderly rows of bristles on the first leg tibia, but on some legs there were many partial rows of three to six bristles (Figure 2D). This was similar to the arrangement seen in sexcombs. The same general pattern was present on the basitarsus. The bractless bristles lack the basal bract which is characteristic of epithelial bristles and were located between the longitudinal bristle row on the leg (Hanna-Alava, 1958). They serve as convenient landmarks, and in this case they illustrate the increased bristle numbers on the legs.

Twenty claw duplications were observed on nineteen feet of six flies (Figure 2A, E). On eighteen of these, only one of the two claws showed a duplication. The other foot had a duplication of both claws. Growths within a tarsal segment (Figure 2E) and on the surface of a tarsal segment (Figure 2F) were present on nine segments. One external growth elongated sufficiently to produce a joint and a more distal tissue mass. It did not form a duplicate tarsal segment. It appeared to be a growth arising from a restricted region of the tarsal segment because it lacked ventral surface bristles indicating a lack of potential to fully duplicate a leg segment. On seventeen of the legs the femur and the tibia were fused on one surface. In ten cases the fusion occurred along more than one-half of the total length of the two segments (see Figure 2A). Both segments appeared to be of normal length and structure except for the fused surface. Twentyfive percent (57/288) of the tarsal segments lacked normal joint development on one surface (Figure 2A, E). Forty one involved the fourth—fifth tarsal joint, and occurred exclusively on the ventral surface. The cuticle was continuous on that surface with no suggestion of invagination to form a joint structure.

Tarsal segments of these legs were of greater diameter than normal. Figure 2A shows the most extreme case in which tarsal segments 2-5 appear spherical rather than cylindrical. The difference was in the diameter rather than

TABLE 1. *The number of selected bristles present on the legs of ci^W/ci^D flies. The mean is based on the number given for each sample (n).*

| | | | Transverse Rows | | | Bractless Bristles[2] Leg | | |
	Sexcomb[1]	Preapical	Apical	Tibia	Basitarsus	1st	2nd	3rd
Normal[3]	12-13	1	1	5.9	7.9	5	5	6
Observed	29.0	1.7	3.0	7.2	9.9	7.0	5.9	8.2
	(4)	(22)	(22)	(18)	(17)	(17)	(15)	(15)

1. Males only
2. Females only
3. Based on Hanna-Alava, 1958.

TABLE 2. *Diameters of segments in arbitrary units (1 unit = 70 μm). The mean and standard deviation are based on the number given for each sample (n).*

	Femur	Tibia	Basitarsus	2nd Tarsus	5th Tarsus
1st leg	2.8 ± 0.6 (16)	1.6 ± 0.5 (17)	0.7 ± 0.2 (19)	0.6 ± 0.1 (18)	0.6 ± 0.1 (18)
2nd leg	2.2 ± 0.4 (16)	1.5 ± 0.4 (17)	0.9 ± 0.3 (18)	0.7 ± 0.3 (14)	0.6 ± 0.1 (16)
3rd leg	2.8 ± 0.5 (9)	2.3 ± 0.5 (13)	1.2 ± 0.4 (18)	1.2 ± 0.3 (16)	0.6 ± 0.1 (17)

the length. The third leg segment had a diameter of 2.2 units compared to 1.2 (Table 2). The length of this segment was not different from the mean length. The lengths were in general agreement with those reported by Hanna-Alava (1958), but as noted by that author there is great variation between stocks so no exact comparison can be made. Table 2 gives the mean diameters of selected leg segments, the notable factor was the high variance, especially on the proximal segments. The second tarsus in Figure 2F showed the same diameter increase. It should be approximately the

same diameter as the third segment. The two males each had one leg on which the three distal tarsal segments were of smaller than normal diameter.

DISCUSSION

Both the wings and the legs of these flies show increased width and structural modifications. The wing blade is broader than normal at the distal end, but this is a characteristic of both ci^W and ci^D so is not an unexpected result. The wing venation pattern is more extreme than either the normal ci^D or ci^W effect, although ci^W may have an effect on all of the veins (Lindsley and Grell, 1968). The third longitudinal vein, the proximal hinge region and the costal veins are uneffected on all of these wings, but every other distal vein, including the marginal vein, is affected. The longitudinal second (L2), fourth and fifth veins are interrupted, or are lacking, while the L2 vein, the first longitudinal vein and the marginal vein are broadened. The anterior marginal bristle pattern is affected by the absence of the triple row bristles on some wings.

The elevated bristle number on the legs is the result of duplication of specific bristles; a multiplication of units as designated by Schubiger (1971). On the transverse rows there are both more bristles per row and extra, short partial rows produced. Tokunaga (1962) has shown that the sexcomb bristles arise form a presumptive transverse row which moves into the longitudinal position observed on the normal leg. The formation of extra transverse bristles in partial rows could account for the increased number and the pattern of the sex comb bristles observed on these flies. The sexcomb is also increased in size in the eyeless-dominant, and in that mutant it appears that adjacent cells are recruited into the sexcomb pattern (Stern and Tokunaga, 1967). That possibility cannot be excluded here since heavy, dark bristles are present immediately adjacent to the sexcomb on both legs of one male.

The bristle duplication cannot be attributed to increased size of the leg segments providing more area for bristle development since many legs which have normal diameters have elevated bristle numbers. The lack of complete joint formation between tarsal segments of normal diameter also suggests that joint development is independent of segment diameter. While many legs of increased diameter have normal joints other leg segments of normal diameter lack joint development on one surface. Bryant and Schneiderman (1969) have shown that the position of joint differentiation is determined by positional reference, so absence of joint formation must reflect the loss of reference on that surface. The presence of extra bristle structures, increased segment diameter and cuticle growths require increased mitotic activity in the region of the duplication (Schubiger, 1971). In the present case this might result because there is a localized loss of reference permitting an increased mitotic activity. The fact that two adjacent enlarged segments can have normal joints suggests that the two factors, mitotic activity and positional reference may be separate. Extra tissue can be produced by increased mitotic activity, yet normal positional reference is indicated by the presence of full joint development.

Schubiger (1971) has constructed a fate map of the leg disc which shows that the leg segments arise from concentric regions of tissue. The most distal segments arise from the most central protion with the most proximal segment arising from the outer portion. The femur and tibia arise from tissue in which the femur producing ring surrounds the tibia producing ring. The fusion of these segments on one surface suggests either a failure of the tissue to differentiate at that point or an increase in mitotic activity which produced more tissue resulting in a fusion. The absence of some wing veins could also result from a loss of positional reference if cells were not properly induced to form the vein. The broadening of vein structures could result if inappropriate cells were induced to produce vein structure or if an increase in mitotic activity resulted in more vein producing cells. The broader wing blade, like the increased diameter of the leg segments, would seem to require increased mitotic activity. As with the legs, it appears that positional reference may be affected and/or there is some influence on mitotic activity. It is not clear whether these are separate actions or in some way interrelated, but it does appear that the activity is localized, possibly confined to specific cellular clones.

Hochman (1973) has presented evidence that ci^W and ci^D are not alleles because $Df(4)M^{63a}$ covers the lethal effect of ci^D but not the phenotypic effects of ci^W. The ci^W/ci^D combination is lethal, with a few individuals surviving to eclose, so it appears that ci^W does not fully cover the lethal effect of ci^D. Stern and Schaeffer (1943) report that as the dosage of ci^W increases (one to three doses) the leg phenotype improves but the wing phenotype becomes more abnormal. The reverse is true as the ci^W dosage decreases. In the present sample no legs as abnormal as those described for $Df(4)M/ci^W$ (shortened and swollen) by Stern and Schaeffer were recovered, but the legs were more modified than those of homozygous ci^W flies. Similar leg aberrations have been observed on presumptive ci^W/O flies obtained from crosses involving compound-fourth chromosomes and on ci^W/O flies which have lost a marked chromosome through induced mitotic loss (unpublishes observations), so it appears that ci^D only partially complements ci^W's effect on leg development. This lack of full complementation suggests that these factors do have interrelated, if not allelic, expressions. Hochman (1973) has suggested that ci^W and ci^D, along with the wing vein mutant Cell-2 (Ce^2; 4-), may be continuous but not strictly allelic. The present evidence does not discriminate between allelic interaction and an epiststic action of two similar dominant factors.

The fact that ci^W has an effect on leg morphology and that legs similar to those observed in these flies have been observed in haplo-ci^W individuals suggests that ci^W has some influence on positional reference in the developing leg, and an influence on local mitotic activity. Whether these are two manifestations of one activity or two separate activities affecting different developmental events is not clear and will have to be distinguished by further studies.

LITERATURE CITED

Bryant, P. J. and H. A. Schneidermen 1969. Cell linage, growth and determination int the imaginal leg discs of Drosophila melanogaster. Dev. Biol. 20:263-290.

Hanna-Alava, A. 1958. Morphology and chaetotaxy of the legs of Drosophila melanogaster. Jour. Morphol. 103:281-310.

Hochman, B. 1973. Analysis of a whole chromosome in Drosophila. Cold Spring Harbor Syp. Quant. Biol. 38:581-589.