

porus, *Scincella*, *Polychrus*, and *Lialis*, and the fish genus *Cyprinodon* (11-13), while the W is derived in the $Z_1Z_1Z_2Z_2/Z_1Z_2W$ system described in the elapid snake *Bungarus caeruleus* (14). The common feature to all of these sex chromosome systems is that the Y or W chromosome is derived, while the homogametic sex presumably represents the ancestral condition.

The distinctive feature in *Staurotypus*, illustrated here in *S. salvinii* and presumably true also for *S. triporcatus*, is that the heterogametic sex is not the most derived. It represents an intermediate condition between the primitive condition, in which the undifferentiated ancestral pair probably resembled the acrocentric male Y and the third group B pair of most other turtles, and the most derived condition in the female having two X chromosomes derived from the autosomal translocation of the secondary constriction and a heterochromatic short arm. Thus, this system does not conform to the general model of sex chromosome evolution for diploid dioecious organisms, in which the Y (or W) becomes heterochromatic and degenerate (15). The Y chromosome is considered to be primitive relative to the X because it is identical in appearance to a pair of homomorphic chromosomes that are widespread in many other turtle genera and families (2, 3, 7). Until more banding data are available for other groups of lower vertebrates, however, we may have no way of knowing precisely what rearrangements have been involved and whether or not the Y (or W) is the only element that has been altered during the evolution of other sex chromosome systems.

JACK W. SITES, JR.
JOHN W. BICKHAM
MIKE W. HAIDUK*

Department of Wildlife and Fisheries
Sciences, Texas A&M University,
College Station 77843

References and Notes

1. J. J. Bull, R. G. Moon, J. M. Legler, *Cytogenet. Cell Genet.* **13**, 419 (1974).
2. J. W. Bickham and R. J. Baker, *Chromosoma* **54**, 201 (1976).
3. J. W. Sites, Jr., J. W. Bickham, M. W. Haiduk, J. B. Iverson, *Copeia* (1979), p. 692.
4. M. Seabright, *Lancet* **1971-II**, 971 (1971).
5. A. T. Sumner, *Exp. Cell Res.* **75**, 304 (1972).
6. S. E. Bloom and C. Goodpasture, *Hum. Genet.* **34**, 199 (1975); C. Goodpasture and S. E. Bloom, *Chromosoma* **53**, 37 (1975).
7. J. W. Bickham, K. A. Bjørndal, M. W. Haiduk, W. E. Rainy, *Copeia*, in press.
8. S. Pathak and A. D. Stock, *Genetics* **78**, 703 (1974).
9. K. Stefos and F. E. Arrighi, *Exp. Cell Res.* **68**, 228 (1971); A. D. Stock, F. E. Arrighi, K. Stefos, *Cytogenet. Cell Genet.* **13**, 410 (1974); W. Becak and M. L. Becak, *Cytogenetics* **8**, 247 (1969); S. Singh, *Chromosoma* **38**, 185 (1972); L. Singh, *Experientia* **28**, 95 (1972); V. G. Ivanov and V. G. Fedorova, *Tsitologiya* **12**, 1582 (1970); M. King and R. Rofo, *Chromosoma* **54**, 75 (1976); M. King and D. King, *Aust. J. Biol. Sci.*

- 28**, 89 (1975); T. R. Chen and A. W. Ebeling, *Copeia* (1968), p. 70.
10. G. C. Gorman and L. Atkins, *Am. Nat.* **100**, 579 (1966); *Copeia* (1968), p. 159; J. J. Bull, *Can. J. Genet. Cytol.* **20**, 205 (1978); C. J. Cole, *Am. Mus. Novit.*, No. 2431 (1970); *Herpetologica* **27**, 1 (1971); *Am. Mus. Novit.*, No. 1653 (1978); L. A. Pennock, D. W. Tinkle, M. W. Shaw, *Cytogenetics* **8**, 9 (1969); T. R. Chen and F. H. Ruddle, *Chromosoma* **29**, 255 (1970).
11. C. I. Cole, *Am. Mus. Novit.*, No. 2450 (1971).
12. J. W. Wright, *Chromosoma* **43**, 101 (1973).
13. G. C. Gorman and L. Atkins, *Syst. Zool.* **16**, 137 (1967); C. J. Cole, C. H. Lowe, J. W. Wright, *Science* **155**, 1028 (1967); G. C. Gorman, R. B. Huey, E. E. Williams, *Breviora*, No. 316 (1969); G. C. Gorman and F. Gress, *Experientia* **26**, 208

(1970); T. Uyeno and R. R. Miller, *Nature (London)* **231**, 452 (1971).

14. L. Singh, T. Sharma, S. P. Ray-Chaudhuri, *Chromosoma* **31**, 386 (1970).
15. J. J. Bull, *Am. Nat.* **112**, 245 (1978).
16. Supported by NSF grant DEB 77-13467 to J.W.B. and Texas Agricultural Experiment Station Project No. 1678 to J. R. Dixon. We thank J. J. Bull for helpful comments, and A. Landazuri-Ortiz of the Direccion General de la Fauna Silvestre, Departamento de Conservacion, for authorizing our work in Mexico.

* Present address: Department of Biological Sciences and The Museum, Texas Tech University, Lubbock 79409.

15 January 1979; revised 24 July 1979

Flatworm Control of Mosquito Larvae in Rice Fields

Abstract. We describe some flatworms (some in the genus *Mesostoma*) that kill mosquito larvae and may account for the variability in the population densities of *Culex tarsalis* and *Anopheles freeborni* in rice fields. When mosquito larvae brush against these worms, the larvae immediately become paralyzed and die. When *C. tarsalis* larvae are placed inside floating cages that exclude flatworms (50-micrometer mesh), there is a fourfold increase in their survival. Rice fields that have abundant mosquito populations lack flatworms. Most such fields have only recently been turned over to rice production, suggesting that the flatworms have difficulty dispersing to new fields but, once established, are able to overwinter and control mosquitoes for the subsequent years of rice production.

The densities of larvae of *Culex tarsalis* and *Anopheles freeborni* from one rice field to another in the Sacramento Valley (1) may differ by two or three orders of magnitude (2). This pattern of distribution is slightly bimodal. Most of the fields have few or no mosquitoes, but a few fields virtually teem with larvae (3). Early investigators thought that toxins produced by blue-green algae might account for these differences, but results from laboratory and field studies have been ambiguous (4). Previously, we found that the survival of larval *C. tarsalis* in screened cages in various rice fields was directly related to the abundance of mosquitoes in the fields at the time of the experiment (3, 5). We have now discovered that rice fields with low densities of mosquito larvae are heavily populated with microscopic flatworms (many under 1 mm in length) that kill the larvae by means of a slimy toxic secretion. These flatworms are able to penetrate the 200- μ m mesh screening that we used in our early field cages. When we placed two flatworms in a 300-ml cup containing 20 second-instar larvae of *C. tarsalis* and observed their activity under a stereoscopic microscope, we noted that the larvae became paralyzed (rigid and motionless) as soon as they contacted one of the worms (Fig. 1). Later, the worms might recontact a paralyzed larva and feed on it, but the worms killed many more larvae than they ate. In fact, all the larvae were killed within 2 hours.

Extensive field sampling and laborato-

ry identification revealed that as many as eight types (species?) of flatworms may be present in the rice fields. The predominant flatworm in these fields was found to be a *Mesostoma* species, a turbellarian in the order Rhabdocoela (6). Certain species in this order possess epidermal rhabdoids (rhabdites) that are slightly curved rods shorter than the height of the epidermis (7). These rods are secreted by gland cells and are discharged to the surface as a toxic mucous secretion that is repellent to predators (8). Although a few literature citations document the toxicity of some flatworm secretions, the extreme potency of the rice field species appears unprecedented (7-9).

We identified 14 rice fields in Sutter, Yuba, and Sacramento counties that differed in aquatic fauna, flora, and physical attributes, and placed four floating cages in each field (10). We stocked the cages with 25 third-instar larvae of *C. tarsalis* bred in the laboratory, and capped each cage with a tight organdy top. In two of the cages we placed 0.2 ml of Tetramin (a commercial fish food used successfully as larval food in the laboratory) every alternate day; the larvae in the other two cages in each field received no supplemental food and served as controls. Each day the dead and live larvae, pupae, and adults were counted, and the dead ones were removed.

After 1 week an average (over all cages in all fields) of 51 percent of the larval mosquitoes had died in the Tetra-

min-containing cages and 53 percent had died in the control cages. In some rice fields, more than 60 percent of the larvae died during the first day. In the laboratory, third-instar mosquitoes can survive without food for up to 3 days, so that food limitation in the field was not indicated. Nevertheless, using two fields in Yuba County that had particularly low larval survival rates, we tested the effect of altering various nutrient levels on larval survival (Table 1).

In each of these two fields we placed 12 experimental and 6 control cages, each cage containing 25 third-instar larvae of *C. tarsalis*. Once every alternate day, the following reagents were added to the experimental cages in each field: (i) 2.5 g of Envirotrol-F, a commercially cultured dry bacteria formulation (11); (ii) a water-soluble tablet containing assorted vitamins (12); (iii) 0.4 g of a plant nutrient formulation containing 2 percent total nitrogen, 9 percent phosphoric acid, and 13 percent soluble potash (13); and (iv) 1.0 g of calcium carbonate. The number of live and dead larvae, pupae, and adults were counted daily and the dead ones were removed. None of these treatments significantly affected larval survival; the average mortality in the experimental cages was between 86 and 94 percent; whereas in the control cages the average mortality was 85 percent.

We then assessed the influence of flatworms on mosquito mortality in the field. Since the flatworms are able to invade the 200- μ m openings in the floating cages, we constructed new cages with windows of only 50- μ m mesh. In each of six rice fields that previously had low larval survival rates (and contained flatworms), we placed three 50- μ m mesh cages and six 200- μ m mesh cages. As before, 25 third-instar larvae of *C. tarsalis* were introduced into each cage and every day for 1 week we counted the number of live and dead larvae, pupae, and adults and removed all the dead.

In every field we found that larval survival in the 50- μ m mesh cages significantly increased (Table 2). Overall, about a fourfold increase in weekly survival was achieved. However, the survival rate in these 50- μ m mesh cages (about 46 percent per week) was substantially lower than the rate we had measured in the 200- μ m cages in some other rice fields (about 90 to 95 percent per week).

Could those mosquito-rich fields with high larval survival rates lack flatworms? To assess the presence or absence of flatworms, we placed three 200- μ m mesh cages in each of 32 rice fields and placed 20 third-instar larvae of *C. tarsalis* in

each cage. After 2 days we inspected the contents of the cages for flatworms. Simultaneously, we assessed mosquito numbers in each field by sweeping 30 times with an aquatic sweep net. Rice fields were categorized according to the number of mosquitoes collected in 30 sweeps: fields with five or fewer mosquitoes, fields with between 6 and 30 mosquitoes, and fields with more than 30 mosquitoes. All developmental stages

and both *Culex* and *Anopheles* species were counted together. We found that no rice field ($N = 11$) in which we found flatworms in the baited floating cages yielded more than five mosquitoes per 30 sweeps. In the fields without flatworms we collected from seven fields less than six mosquitoes, from eight fields between 6 and 30 mosquitoes, and from six fields more than 30 mosquitoes per 30 sweeps (14). A χ^2 contingency analysis

Table 1. The effect of selected nutrients on the survival (percentage survival per week) of *C. tarsalis* larvae (25 larvae per cage) in floating cages. The compounds were placed twice daily in the cages in two rice fields with particularly low larval survival rates in earlier experiments.

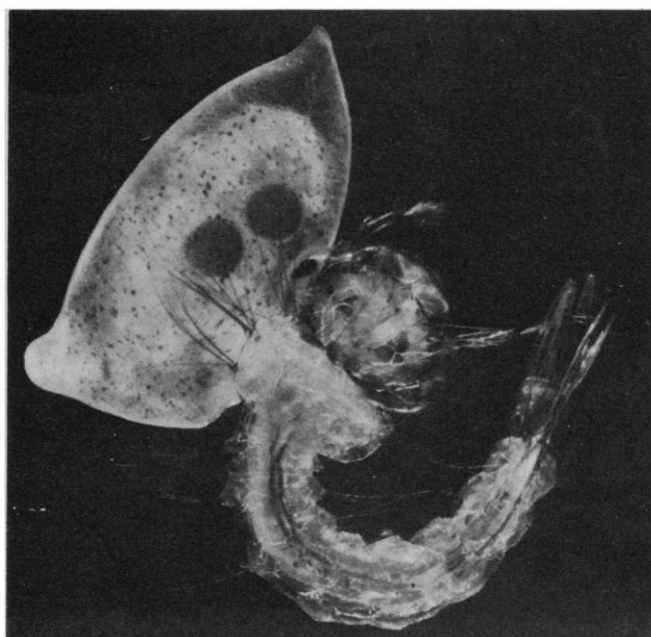
Treatment	Percentage survival (mean)	Two standard errors	Number of cages
Bacteria culture	8	7.8	6
Vitamins	14	24.0	6
Calcium carbonate	6	13.8	6
Plant nutrients	10	9.0	6
Controls*	15	20.6	12

*No supplemental nutrients provided.

Table 2. Mean percentage survival per week for 25 larvae of *C. tarsalis* introduced into floating cages placed in various rice fields that contained flatworms. The cages had windows that were screened with either a 200- μ m mesh organdy or a 50- μ m mesh plastic fabric. Only the latter excluded the flatworms.

Field	Cages with 200- μ m mesh ($N = 6$)		Cages with 50- μ m mesh ($N = 3$)	
	Percentage survival (mean)	Two standard errors	Percentage survival (mean)	Two standard errors
A1	13	8.4	39	22.0
A3	13	11.4	35	16.7
B1	1	1.2	51	24.3
B2	24	11.8	51	22.8
B5	7	9.5	78	11.0
B9	14	4.6	24	18.2
Grand mean	12		46	

Fig. 1. Photograph ($\times 48$) of a flatworm (*Meisostoma* sp.) from a California rice field killing a *Culex tarsalis* mosquito larva. (Photo by J. K. Clark, University of California, Davis.)



of these results revealed that the negative association between flatworms and mosquitoes could not be explained by chance alone [$\chi^2(2) = 13.3$, $P < .05$]. The seven rice fields that lacked flatworms but still yielded few (less than six) mosquitoes either had been stocked with the mosquito fish *Gambusia affinis* or were cool-water fields with a summer midday temperature usually below 21°C (3).

Our data show that rice fields with large numbers of flatworms produce few mosquitoes, even if the fields contain warm water and are not stocked with *Gambusia*, and that in the laboratory the flatworms are directly responsible for the death of larvae of *C. tarsalis*. Exclusion of the flatworms by the floating field cages causes a fourfold increase in larval survival but survival under these conditions is still lower than that in other fields that lack flatworms altogether. Perhaps in fields infested with flatworms the toxin is able to diffuse into the floating cages in sufficient quantities to adversely affect the mosquito larvae. An alternative explanation is that some other micro-organism in flatworm-infested fields may also be detrimental to mosquitoes but may lack virulence under our laboratory conditions (3).

Also puzzling is the question of why some rice fields support flatworms and others do not. Most of the fields in this study that had inordinate numbers of mosquitoes and lacked flatworms were fields that had just been turned over to rice production. Typically, farmers in the Sacramento Valley will rotate rice to safflower or some other crop every 3 to 5 years. Perhaps flatworms have difficulty dispersing to new rice fields but once established they are able to overwinter and control mosquitoes for the successive years the field is in rice. Further study should reveal the validity of this hypothesis.

TED J. CASE

Department of Biology,
University of California, San Diego,
La Jolla 92093

ROBERT K. WASHINO

Department of Entomology,
University of California, Davis,
Davis 95616

References and Notes

1. In the Sacramento Valley of California, typically over 400,000 acres of land are devoted annually to rice production. These fields are usually flooded in early May. When the rice emerges, populations of larval *Culex tarsalis* develop in the warm water and usually reach a peak abundance in mid-June. By July or August the rice increasingly shades and cools the water and populations of *Anopheles freeborni* then predominate. Both *C. tarsalis* and *A. freeborni* are serious pests and pose a threat to public health

- as vectors of Western equine and St. Louis encephalitis and malaria, respectively [S. F. Bailey and P. A. Giecke, *Proc. Calif. Mosq. Control Assoc.* 6, 53 (1968)].
2. D. F. Womeldorf and K. G. Whitesell, *Mosq. News* 32, 364 (1972); R. K. Washino, K. G. Whitesell, E. J. Sherman, M. C. Kramer, R. J. McKenna, *ibid.*, p. 375; W. Ahmed, R. K. Washino, P. A. Giecke, *Proc. Calif. Mosq. Control Assoc.* 38, 95 (1970); W. C. Purdy, *U.S. Public Health Serv. Public Health Bull. No. 145* (1924).
3. T. J. Case and R. K. Washino, in preparation.
4. R. W. Gerhardt, *Proc. Calif. Mosq. Control Assoc.* 22, 50 (1953); *ibid.* 23, 120 (1955); *ibid.* 24, 47 (1956); R. Matheson and E. H. Hinman, *Am. J. Hyg.* 8, 279 (1928); *ibid.* 11, 174 (1930); M. E. MacGregor, *Parasitology* 12, 382 (1924); S. V. Amonkar, thesis, University of California, Riverside (1969).
5. T. J. Case and R. K. Washino, *Proc. Calif. Mosq. Control Assoc.* 44, 155 (1975).
6. F. H. Collins and R. K. Washino, *ibid.* 46, 91 (1978).
7. L. H. Hyman, *The Invertebrates* (McGraw-Hill, New York, 1951), vol. 2.
8. S. J. Coward and J. W. Piedilato, *J. Biol. Psychol.* 15, 5 (1973).
9. R. A. Medved and E. F. Legner, *Environ. Entomol.* 3, 637 (1974).
10. The floating emergence cages were constructed from 1-gallon size plastic buckets with organdy screen windows (approximate mesh size 200 μ m) on the sides and bottoms. Three fishing bobbers were placed near the top to keep the buckets afloat.

11. Available from Mardel Laboratories, Coral Stream, Ill.
12. Available from Aquarium Pharmaceuticals, Perkasie, Pa. Each tablet contains 120 mg of thiamine, 0.10 mg of riboflavin, 2.0 mg of niacin, 0.20 mg of pyridoxine, 1.50 mg of pantothenic acid, 0.003 mg of biotin, 0.20 mg of folic acid, 0.025 mg of aspartic acid, 0.025 mg of glutamic acid, 0.03 mg of choline, and 0.02 mg of inositol.
13. Aquarium plant food, Aquarium Pharmaceuticals, Perkasie, Pa.
14. As an independent check on flatworm occurrence, micro-rice field ecosystems were made by filling 1-gallon plastic aquariums with soil, algae, macrophytic plants, and unsieved water samples from each of the fields. A 300-ml plastic cup with 200- μ m mesh windows was floated on each aquarium. Fifteen third-instar *C. tarsalis* were placed in each cup and the presence or absence of flatworms in the cup was noted after 1 week. In all but 2 of the 32 fields these two methods gave the same results concerning the presence or absence of flatworms. For the two fields for which the results differed, flatworms were found in the field but not in the laboratory aquariums.
15. We thank the following persons for their assistance: C. K. Fukushima, K. Luchessa, M. F. Feldlaufer, R. P. Meyer, T. McKenzie, R. Dunn, M. Lee, K. G. Whitesell, and the Colusa Mosquito Abatement District. This work was supported by a special University of California Augmentation for Mosquito Control Research.

12 April 1979; revised 18 June 1979

Vascular Smooth Muscle: Aerobic Glycolysis Linked to Sodium and Potassium Transport Processes

Abstract. Under aerobic conditions, glucose is primarily catabolized by vascular smooth muscle to lactate, in spite of an adequate oxidative capacity. Although this is often considered to be indicative of some nonspecific metabolic insufficiency, there is evidence that aerobic glycolysis is specifically coupled to sodium and potassium transport processes, whereas oxidative metabolism is coupled to contractile energy requirements.

Under fully oxygenated conditions, lactic acid is a major end product of vascular smooth muscle (VSM) metabolism. Such aerobic glycolysis has long been an enigma since it is normally associated with relatively few cell types, such as tumor or retinal cells, which generally are poorly oxygenated in comparison with VSM (1). Although it is sometimes con-

sidered to be an artifact of tissue preparation (2), substantial aerobic glycolysis is a consistent finding in reports of vascular metabolism and indeed of smooth muscle in general (3). The mismatch of glycolytic and oxidative metabolism in VSM (50 to 90 percent of the glucose entering the cell is lost as lactate) has been the source of considerable speculation.

Table 1. Steady-state values of metabolic and contractile parameters in porcine coronary arteries. Measurements were made during the second 1/2 hour of the experimental period. Metabolic functions are expressed as a fraction of the values obtained in the absence of stimulation: $J_{O_2} = 0.095 \pm 0.006$ μ mole/min-g ($N = 29$); $J_{lac} = 0.150 \pm 0.006$ μ mole/min-g ($N = 77$). Active isometric force (ΔP_0) is expressed as a fraction of that induced by 80 mM KCl. Contraction induced by the pharmacological agonist histamine is included for comparison. Number of tissue measurements is included in parentheses after each value.

Condition	J_{lac}	J_{O_2}	ΔP_0
KCl*	1.67 ± 0.08 (28)	1.68 ± 0.07 (23)	1
K ⁺ substituted for Na ⁺	0.28 ± 0.05 (8)	1.93 (1)	1.88 ± 0.14 (3)
K ⁺ substituted for Na ⁺ + 50 mM sucrose	0.34 ± 0.03 (8)	1.57 (2)	1.60 ± 0.19 (3)
K ₂ SO ₄ substituted for NaCl	0.60 ± 0.05 (12)	1.66 ± 0.12 (7)	1.50 ± 0.10 (4)
Ouabain (10^{-5} M)	0.53 ± 0.05 (12)	1.21 ± 0.035 (11)	0.62 ± 0.14 (10)
K ⁺ -free PSS	0.54 ± 0.04 (10)	$.99 \pm 0.09$ (9)	0.22 ± 0.07 (7)
K ⁺ restored	1.18 ± 0.17 (6)	1.04 ± 0.09 (7)	-0.02 ± 0.07 (6)
Histamine (10^{-4} M)	1.85 ± 0.12 (8)	1.84 ± 0.12 (7)	1.51 ± 0.31 (5)

*Added to cause an 80 mM increase in bath concentration.