mented together with ends staggered and pulled to a total tip diameter of 5 to 6 μm. The recording barrel was filled with 5*M* NaCl. The barrels to be used for pressure ejection were filled with 750 m*M* ethanol in normal saline, with normal saline alone, or with 670 m*M* sucrose in normal saline, which is isosmotic with the ethanol solution.

10. For pressure ejection of ethanol, nitrogen gas at 100 psi was connected to a pressure ejection control unit (Medical Systems), permitting precise control of the amplitude and time of pressure applied to the ethanol-containing pipette barrels. All micropressure ejection doses are given as pounds per square inch times seconds. Recent investigations with micropressure ejection have shown that release of drug volume is reproducible and is linearly related to both time and pressure [R. F. McCaman, D. G. McKenna, J. K. Ono, *Brain Res.* **136**, 141 (1977); M. Sakai, B. Swartz, C. Woody, *Neuropharmacology* **18**, 209 (1979); M. Palmer, S. Wuerthele, B. Hoffer, in preparation]. Furthermore, these studies suggest that the pressure and time parameters can be interchanged (that is, 5 psi × 10 seconds equals 10 psi × 5 seconds) without significantly altering the volume of drug released, and also suggest that there is little drug leakage between ejection trials. In addition, these studies show that the volume ejected in a 50 psi-second pulse is on the order of 10^{-5} μl, so that even though there is an ethanol concentration of 750 m*M* in the micropipette barrel, the concentration at the cell membrane is far less.
11. The rate of spontaneous discharge of Purkinje cells was integrated over 1-second intervals and displayed as spikes per second on an inkwriter. These ratemeter records were subsequently digitized on a digitizing tablet interfaced with a Data General NOVA 3/12 computer. With appropriate software, it was possible to determine the average discharge rate before, during, and after ethanol administration and to compute the percent changes. To be used for analysis, all single-unit recordings had to show stable patterns during the control period, consistent changes after ethanol administration, and subsequent recoveries of stable patterns. This usually required continuous recording for 1 to 3 hours.
12. If a response is supramaximal, the question of whether a smaller dose of ethanol would give the same response arises. If a response is just threshold or subthreshold, it is conceivable that a larger dose of ethanol could elicit an effect of similar magnitude. Hence, after determinations of dose-response relationships for each Purkinje cell, the dose giving a 30 to 70 percent response was selected because it was certain that larger and smaller doses would elicit greater and lesser effects, respectively. Overall, however, the mean depression in the firing rate of the population of LS Purkinje cells produced by an average dose of 29 psi-seconds was 54 percent, and the mean depression in firing rate for the population of SS Purkinje cells produced by an average dose of 888 psi-seconds was 52 percent. There was no significant difference in these mean population depressions.
13. The question of long-term tolerance has been extensively reviewed [D. B. Goldstein, *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **39**, 1943 (1975); E. Majchrowicz, *Psychopharmacologia* **43**, 245 (1975)] and has been seen electrophysiologically in the hippocampal slice in vitro [W. A. Corrigal and P. L. Carlen, *Proc. Annu. Meet. Soc. Neurosci 8th.* (1978), Abstr. 1329]. It has been shown that SS mice develop tolerance to the hypnotic effects of alcohol much more rapidly than LS mice do, with a regimen of multiple injections of ethanol [B. Tabakoff, R. Ritzman, T. Raju, R. Deitrich, *Alcoholism Clin. Exp. Res.* **4**, 70 (1980)]. Short-term tolerance to ethanol has also been described with respect to motor behavior [A. E. LeBlanc, H. Kalant, R. Gibbons, *Psycholpharmacologia* **41**, 43 (1975)], nystagmus [I. A. Mirsky, P. Piker, M. Rosenblum, M. Lederer, *Q. J. Stud. Alcohol* **2**, 35 (1941)], and EEG phenomena [L. V. Story, E. Eidelberg, J. French, *Arch. Neurol.* **5**, 565 (1961)].
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15. Supported by PHS grants AA-03527 and GM-01983. M. Palmer has a fellowship from the Pharmaceutical Manufacturers' Association and a scholarship from the Reiger Educational Trust. We thank R. A. Dietrich and R. Freedman for critical reading of the manuscript.

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Osmolality and Potassium Ion: Their Roles in Initiation of Sperm Motility in Teleosts

Abstract. *Spermatozoa that are quiescent in electrolyte and nonelectrolyte solutions isotonic to seminal plasma show motility when the semen is diluted with hypotonic solution in freshwater teleosts (four species tested) and with hypertonic solution in marine teleosts (five species tested). Decrease or increase, respectively, in osmolality of the environment may be the factor initiating sperm motility in these species. The motility of chum salmon spermatozoa in a sodium chloride solution isotonic to seminal plasma is completely suppressed by approximately 10 millimoles of potassium per kilogram; topminnow spermatozoa, however, were immotile in a nonelectrolyte solution, and motility was induced by electrolytes, especially potassium. Thus ions, rather than osmolality, may be an essential determinant of sperm motility in salmonid and viviparous teleosts.*

In many animals, hypotonicity is harmful to sperm structure and sperm motility. Sperm heads of marine invertebrates swell immediately after immersion in media hypotonic to seawater. Diluted Ringer solution causes the sperm tails of mammals to spiral and cease motility (*1*). In salmonids, the life span of spermatozoa is longer in 20 percent seawater than in freshwater (*2*). In teleosts, spermatozoa that are immotile when normally suspended in a seminal plasma having an osmolality of about 300 to 350 mosmole/kg in freshwater or marine species (*3*) or containing high concentrations of potassium ion in Salmonidae (*4*) become exposed at spawning to environments that are hypotonic, hypertonic, or low in K^+, respectively.

In a freshwater teleost, the goldfish *Carassius auratus*, and in a marine teleost, the puffer *Fugu niphobles*, the sperm were immotile when the semen (*5*) was mixed with 100 to 200 volumes of either an electrolyte (150 mmole/kg NaCl or KCl) or a nonelectrolyte (300 mmole/kg mannitol) solution isotonic to the seminal plasma (300 mosmole/kg) (Fig. 1, a and b). Motility occurred only when the semen was diluted with a solution (electrolyte or nonelectrolyte) of lower osmolality for the freshwater species or higher osmolality for the marine species. Thus, the experimental conditions parallel the natural conditions in which sperm motility is suppressed by the osmolality isotonic to the seminal plasma in the testis and initiated by a decrease or an increase in the osmolality of the environment surrounding the spermatozoa at spawning. Duration of sperm motility in the goldfish increased with a decrease in osmolality in both NaCl and mannitol solutions, reaching a maximum motility at 100 mosmole/kg before decreasing. This decrease in amount and duration of sperm motility is probably the result of disruption of sperm structure by hypoosmotic shock, since swollen sperm heads and fragmented tails were noted. Because spermatozoa have a longer life span at an osmolality between 100 and 200 mosmole/kg, they must reach spawned oocytes within a short period in an environment constituted by the mixture of seminal plasma and freshwater. In fact, the duration of sperm motility of teleosts—including goldfish—is relatively short, and generally the mature male of many fishes approaches the female and releases spermatozoa immediately after oviposition. In the puffer, the duration of sperm motility reached a maximum at 400 mosmole/kg and then decreased with further increase in osmolality. The spermatozoa ceased to move at osmolalities (1200 mosmole/kg) above that of seawater (1000 mosmole/kg).

Sperm motility was induced in other freshwater teleosts—*Cyprinus carpio, Carassius carassius*, and *Tribolodon hakonensis*—by reducing the osmolality, and in other marine teleosts—the cod *Gadus morrhua macrocephalus* and the flounders *Limanda yokohamae, Microstomus achne*, and *Kareius bicoloratus*—by increasing the osmolality. In the seawater teleosts, sperm motility ceased at an osmolality greater than 1200 mosmole/kg.

Spermatozoa of the chum salmon *Oncorhynchus keta*, which belongs to a primitive group of Teleostei, the Salmonidae, were motile in NaCl solutions between 0 and 500 mosmole/kg (Fig. 1c); osmolalities of 300 to 400 mosmole/kg, which are similar to that of the seminal plasma of chum salmon (*4*), did not inhibit motility. The seminal plasma of the rainbow trout *Salmo gairdnerii* contains a high concentration of K^+, which inhibits sperm motility, and K^+ is also a major constituent of the seminal plasma of chum salmon (*4*). In our study, sperm motility in chum salmon and rainbow trout (*6*) did not occur at any KCl concentration examined. When ion concentration was increased by adding various concentrations of KCl to 100 mmole/kg NaCl, sperm motility ceased, although

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vigorous motility continued in the NaCl solution at concentrations above 100 mmole/kg; 10 mmole/kg KCl was sufficient to inhibit sperm motility. Sperm motility is apparently suppressed by K^+ (about 80 m*M*) in the testis and sperm duct of Salmonidae, and the reduction of K^+ concentration at spawning induces vigorous movement of the spermatozoa.

Breakdown of the sperm ball (*7*) of the viviparous topminnow *Gambusia affinis* (Fig. 1d), as well as that of the guppy, occurred in NaCl or KCl solutions at concentrations between 50 and 300 mmole/kg. Since the spermatozoa in the sperm ball of the topminnow and guppy begin to move 20 to 30 seconds after immersion in electrolyte solutions, and this is followed by breakdown of the sperm ball, potassium and sodium can be considered as having a direct stimulating effect on sperm motility. Sperm ball breakdown did not occur in a nonelectrolyte solution such as mannitol. Since the spermatozoa of viviparous teleosts enter ovarian fluid that may have an osmolality similar to that of seminal plasma, it is likely that osmolality is not a factor inducing sperm motility in the topminnow. A change in ionic concentrations, especially in the concentration of K^+, which induces complete sperm ball breakdown, may participate in the initiation of sperm motility in the ovary of viviparous teleosts.

Physical constraint (*8*), low O_2 tension (*9*) or high CO_2 tension (*10*), and *p*H lower than that of seawater (*11*) have been proposed as the factors responsible for quiescence of spermatozoa in the testis of marine invertebrates. Release from these factors, as well as exposure to heavy metals such as copper and zinc, which are contained in seawater (*12*), has been thought to cause sperm motility. These factors, although they have complicated effects both on sperm motility and respiration, may prove to be operative in induction of sperm motility in invertebrates by causing an increase in intracellular *p*H through exchange of Na^+ and H^+ (*13*). This seems likely inasmuch as simple factors (*14*) such as osmolality in freshwater and marine species and K^+ concentration in salmonids and probably in viviparous species are concerned with the initiation of sperm motility in teleosts.

The contribution of the osmotic or ionic environment to sperm motility in oviparous teleosts, as well as the lack of sensitivity of the spermatozoa of viviparous teleosts to osmolality, may reflect adaptation of fish spermatozoa to the particular environment in which they are spawned. This is an interesting consideration in regard to evolution of the Teleostei, which have undergone adaptive radiation by successfully coping with the osmotic difference between their body fluid and their environment.

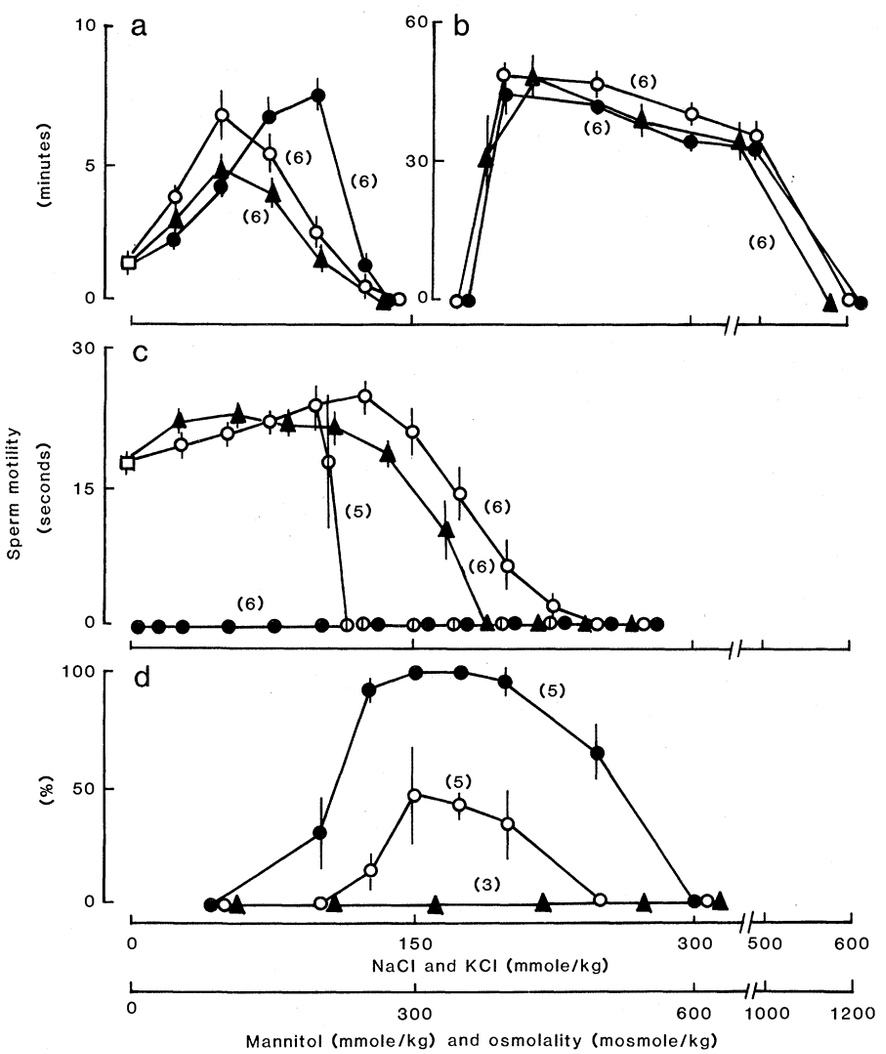

Fig. 1. The effects of Na^+, K^+, and mannitol on spermatozoan motility of (a) goldfish, (b) puffer, (c) chum salmon, and (d) topminnow. Sperm motility of goldfish, puffer, and chum salmon was determined by measuring the time until all spermatozoa ceased to move when observed with light microscopy. Sperm ball breakdown is indicated as the percentage of sperm balls that were completely disrupted 15 minutes after immersion in the test solutions (sperm ball breakdown reached a plateau by this time). Symbols indicate 10 m*M* Hepes buffer (□), NaCl (○), KCl (●), mannitol (▲), and 100 mmole/kg NaCl plus various concentrations of KCl (◐). Numbers of experiments are indicated in parentheses. Values are means ± standard errors.

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5. The semen was collected carefully by gently pressing the abdomen of the teleosts and immediately diluted with the medium containing various concentrations of NaCl, KCl, and mannitol, buffered with 10 m*M* Hepes-NaOH at *p*H 7.7 [Hepes: 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid]. In order to cause goldfish spermiation, human chorionic gonadotrophin (Teikokuzoki) was injected intraperitoneally 24 or 48 hours earlier at a dose of 50 I.U. per 10 g of body weight.
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7. In some viviparous teleosts, a large number of spermatozoa aggregate to form a so-called sperm ball. In the guppy *Poecilia reticulata*, the sperm ball disintegrates in the ovary within 3 hours after copulation, and sperm motility results (K. Takano, personal communication).
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14. In our observations in the goldfish, no sperm motility occurred by reduction in sperm density after addition of sufficient isotonic solution to increase volume by as much as 800-fold, or by introduction of air or oxygen for 1 hour in isotonic medium, or by incubation in isotonic solution at pH 7.7, which is the optimum for continued sperm motility.
15. We thank H. A. Bern, University of California, Berkeley, and H. Mohri, University of Tokyo, for reading this manuscript and the director and staff of the Misaki Marine Biological Station of the University of Tokyo and of the Otsuchi Marine Research Center, Ocean Research Institute, University of Tokyo, for providing the materials. Supported in part by grants-in-aid from the Japanese Ministry of Education.

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Antbutterflies: Butterflies That Follow Army Ants to Feed on Antbird Droppings

Abstract. *Females of three species of tropical rain forest ithomiine butterflies orient to swarms of army ants (*Eciton burchelli*) and feed on bird droppings found there. The antbirds associated with swarm raids of these ants provide a predictable source of droppings, an otherwise sparsely distributed resource.*

Females of three butterfly species of the Ithomiinae (Lepidoptera: Nymphalidae) are associated with raiding swarms of the army ant *Eciton burchelli* (Hymenoptera: Formicidae) and feed on bird droppings in the swarm vicinity. Many species of birds, primarily in the antbird family, Formicariidae, deposit droppings as they follow swarms of *E. burchelli* to feed on insects flushed from the leaf litter by the ants (*1*). Thus army ant swarms provide a predictable source of nutrients as bird droppings, and the odors associated with the swarms may allow the butterflies to orient to the location of fresh droppings to feed.

As a result of an initial observation that, at Finca La Selva in Costa Rica (*2*), females of three species of ithomiines (*Mechanitis polymnia isthmia, Mechanitis lysimnia doryssus*, and *Melinaea lilis imitata*) assembled in high densities at *E. burchelli* swarms and fed on bird droppings there, we undertook a 1-year study of the behavior, sex ratios, and turnover within such assemblages. From these data we sought clues as to the nature of associations between the ants, birds, and butterflies.

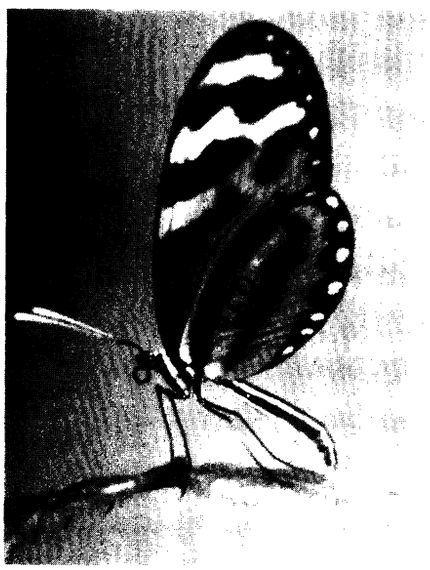

Fig. 1. Adult of *Mechanitis lysimnia doryssus* laying eggs on its *Solanum* host plant at Finca La Selva, Costa Rica. Larvae feed in groups and sequester poisonous compounds from the leaves, which provide protection from predation by antbirds, as adults.

Nonfloral feeding by adult Lepidoptera is a poorly explored phenomenon (*3*) although it can have significant effects on reproductive biology. Reproduction in most kinds of butterflies is thought to be restricted by the amount of nitrogenous reserves accumulated during larval feeding (*4, 5*). Many butterflies, particularly temperate butterflies, feed primarily from flowers and thereby obtain sugar to provide metabolic energy. For these butterflies, larval reserves are the major, if not only, source of nitrogenous materials available for egg production. When the butterfly emerges from the pupal case, the number of eggs to be laid is thought to be a finite number determined by the quantity of nutrients accumulated during larval feeding (*4, 6, 7*). This general life pattern does not hold for several groups of butterflies. Adult *Heliconius* (Nymphalidae: Heliconiinae) butterflies use free amino acids in pollen to augment egg production, thereby extending their reproductive life (*8*). Other Neotropical butterflies, including the subfamily Ithomiinae, feed on bird droppings, and it is believed that they are using uric acid or partly digested proteins as a source of nutrients essential for egg production (*6, 8, 9*). Some ithomiine species are able to live at least 4 months (*8*), and probably produce egg clusters (Fig. 1) throughout their lives (*10*). If ithomiines depend on bird droppings as a nutritional resource necessary for prolonged egg production, then selection should favor efficient exploitation of predictable sources of fresh bird droppings. Raiding swarms of the army ant *E. burchelli* are such a predictable source.

At Finca La Selva, two other types of adult ithomiine groupings are known to occur. Male butterflies may be found in high densities at certain species of plants which provide precursors for the sex pheromone [ithomiine males bear scent scales which emit the sex pheromone (*3, 11, 12*)]. In addition, there are courtship aggregations composed mainly of males. Both males and females are attracted to the courtship area (*12*); however, the sex ratio is strongly shifted toward males (Table 1). At ant swarms we find the only ithomiine aggregations with female-biased sex ratios (Table 1).

At dense ant swarms, a large contingent of female butterflies, approximately 8 to 12 at one time, may be observed flying low over the front of the swarm. As many as 30 individuals have been captured over a period of a few hours. It is possible that many more individuals may be present at the swarm, since it is difficult to see and capture butterflies in a dense forest habitat. The butterflies exhibit searching behavior, dipping down momentarily in the midst of the dense swarm, and occasionally stop to feed at fresh bird droppings.

It appears that the butterflies are attracted from a large area to the swarms, possibly by some odor produced by the

Table 1. Sex ratios (male:female).

Species	Pheromone plants	Courtship groups	Ant swarms	At emergence from pupae
M. polymnia	36:1	28:13	10:99	60:64
M. lysimnia	1:0	22:4	2:40	
M. lilis	1:0	3:2	5:31	1:5

Table 2. Abundances.

Species and sex	Individuals (number per hour)		Probability‡
	Ant* swarms	Without ants†	
M. polymnia ♀	2.65	.256	.001
M. polymnia ♂	.267	.341	.4
M. lysimnia ♀	1.07	.085	.01
M. lysimnia ♂	.054	.085	.3
M. lilis ♀	.829	0	8×10^{-5}
M. lilis ♂	.134	.085	.3

*Thirteen swarms, for a total of 37.4 hours. †Five sessions, for a total of 11.7 hours. ‡Computed by means of a *t*-test. Each swarm or session is treated as one datum; these are used to compute the mean, weighted by the length of the session.

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