the HPRT enzyme when hypoxanthine is present.

The growth rate of control and Lesch-Nyhan cells in tissue culture is the same (*9*). Presumably, net total synthesis of RNA and DNA by the two cell types is the same. Lesch-Nyhan cells, with almost no HPRT activity, require a mechanism to generate a higher concentration of PRPP because PRPP amidotransferase has a higher K_m for PRPP than HPRT.

Experiments with the HPRT kinetic mutant cells are interesting in this regard. The K_m of the mutant HPRT for PRPP is 1 m*M* (*9*) which is more than twice that of control PRPP amidotransferase (*15*). In C.M. cells, PRPP is utilized by PRPP amidotransferase and there is little function of the mutant HPRT (*11*). We have previously shown that these cells utilize mutant HPRT in the presence of aminopterin (*11*). In C.M. cells, PRPP synthetase activity rises to a new steady state (Figs. 1A and 3), and this generates the PRPP concentration shown in Fig. 2A, which in turn enables these cells to grow at normal rates in tissue culture (*9*). Pathways in purine metabolism are shown in Fig. 5.

These experiments do not examine the mechanism of PRPP and PRPP synthetase changes, or the relative importance of PRPP synthetase regulation versus PRPP amidotransferase regulation in the control of de novo purine synthesis. Most studies have been done with partially purified enzyme preparations and not with whole cells under physiologic conditions (*15*). Both regulatory enzymes may be important in the fine regulation of purine metabolism. It is interesting that the two enzymes have different relative responses to mono- and trinucleotide inhibitors and inorganic phosphate (*4, 15*). That there may be a dual regulation of purine metabolism can be inferred from the studies of Becker (*17*), who found that cells grown in inosine (hypoxanthine riboside) demonstrate an increase in PRPP at the same time that de novo purine synthesis is inhibited, presumably by nucleotide effects on PRPP amidotransferase. The studies presented here do suggest the existence of mechanisms that adjust the concentrations of cellular PRPP to those required for purine needs and cellular growth. PRPP levels depend on whether the cell utilizes HPRT, PRPP amidotransferase, or a mutant HPRT activity to obtain its purine requirements.

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13. The PRPP synthetase was assayed by a modification of the two-step procedure of A. Hershko, A. Razin, and J. Mager [*Biochim. Biophys. Acta* **184**, 64 (1969)]. Fibroblasts were disrupted by freezing and thawing three times; they were then mixed with Norit A charcoal, 10 mg/ml in 1 m*M* EDTA. The charcoal and cellular debris were removed by centrifugation at 10,000*g* for 15 minutes. The reaction mixture for step one consisted of 50 m*M* tris buffer, *p*H 7.4, 5 m*M* $MgSO_4$, 5 m*M* glutathione, 1 m*M* EDTA, 50 m*M* Na_2HPO_4, 0.1 m*M* adenosine triphosphate, 0.1 m*M* ribose-5-phosphate, and cell extract in a final volume of 0.25 ml. The PRPP formed in this step and the content of fibroblast extracts were assayed by a modification of the method of J. F. Henderson and M. K. Y. Khoo [*J. Biol. Chem.* **240**, 2349 (1965)]. The reaction mixture consisted of 55 m*M* tris buffer, *p*H 7.4, 5 m*M* $MgCl_2$, 10 nmole of [^{14}C]hypoxanthine (50.4 mc/mmole), cell extract, and partially purified HPRc in a total volume of 0.125 ml. The [^{14}C]inosine monophosphate formed was isolated by high-voltage electrophoresis in 0.05*M* sodium borate buffer, *p*H 9.0, and the addition of appropriate carrier compounds. For the PRPP synthetase assay, background PRPP levels were subtracted by using a blank reaction mixture which contained no ribose-5-phosphate.
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Aggressive Chemical Mimicry by a Bolas Spider

Abstract. *Mature female* Mastophora *sp. spiders attract prey with a volatile substance which apparently mimics the female sex attractant pheromone of the fall armyworm* Spodoptera frugiperda *(Lepidoptera). The rate of prey capture is similar to that of a conventional orb weaver of comparable body size.*

Bolas spiders of the genus *Mastophora* are descended from orb weavers, but their webs have become reduced to a sticky ball suspended on the end of a short vertical thread that is attached to a single horizontal line (*1*). They are found throughout the Americas, but, perhaps because of their cryptic habits, are rare in collections. Previous observations have shown that the spider hangs on the horizontal thread, and holds the vertical thread with one front leg (Fig. 1A), swinging the ball at passing insects. When the ball hits an insect, it sticks, and the spider then descends the line, paralyzes the prey, and feeds (*1*). Whether or not the spider is able to attract its prey from a distance has been uncertain (*2*). Evidence is presented here showing that this improbable and seemingly ineffective trapping method derives its success in *Mastophora* sp. (*3*) from the use of volatile substances that apparently mimic prey sex attractant pheromone (*4*).

Several types of evidence suggest the use of a chemical attractant. The prey always approached slowly from directly downwind of the spider (more than 100 observations) with their antennae extended (Fig. 1E), and often they made repeated passes at the spider. Occasionally it was possible to follow their flight several meters from the spider: typically the animal slipped 10 m or so downwind of the spider, made several wide and erratic arcs there, and began moving upwind, narrowing the arcs as it neared the spider. When a cardboard sheet was held just downwind of the spider to deflect the airflow and hide the spider from sight, prey continued to approach, arriving by way of the trailing edge (Fig. 2).

That prey were attracted to the spider rather than the ball was shown by experimentally removing the ball just after it

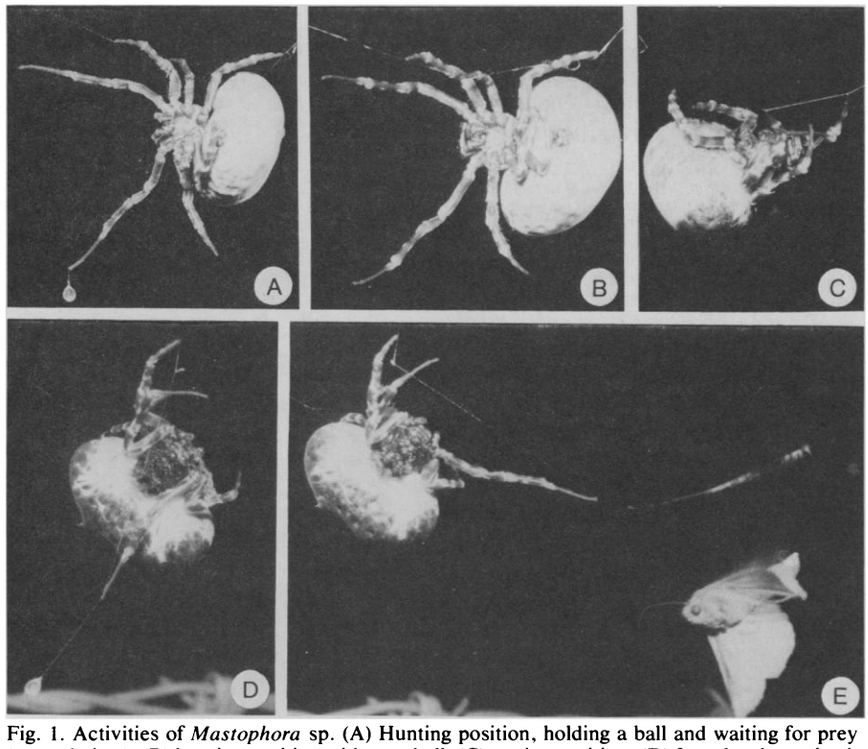

Fig. 1. Activities of *Mastophora* sp. (A) Hunting position, holding a ball and waiting for prey (ventral view); (B) hunting position without a ball; (C) resting position; (D) front leg drawn back ready to swing the ball (front view); (E) swinging ball (which elongates reversibly) at a nearby moth. Spider's abdomen is about 1.3 cm wide.

was made (*5*). The spider usually assumed its hunting stance without the ball (Fig. 1B), and the ball was then placed on a nearby piece of filter paper. In four such experiments, no prey approached the filter paper, and 14 approached the ball-less spider (*6*). Unmanipulated spiders also occasionally assumed predatory stances without balls. With only one exception (*7*), prey only approached when the spider was in its hunting position (with or without a ball), and never when it was resting (Fig. 1C), suggesting that the spider only releases attractive substance when hunting.

The spiders' behavior was appropriate for prey approaches from downwind. The movement used to swing the bolas was a very quick ventral flexion of the front leg, and the spider usually adjusted its position so that its ventral side faced downwind. Prey approaching ventrally were probably not perceived visually since the spider's eyes are dorsal, but the spiders consistently responded by extending their front legs laterally when I made sudden humming noises, and they may thus sense the prey's approach at least partially by the sound it makes.

Of 165 prey collected after a spider had fed on them (*8*), all were male noctuid moths of two species. In one series of nights, one spider caught 15 *Spodoptera frugiperda* and 13 *Leucania* sp. (*9*), while the others caught 66 *S. frugiperda*. An additional 28 prey collected as they hovered just downwind of a spider were also all male moths: 23 *S. frugiperda*, 4 *Leucania* sp. (all near the same spider which captured *Leucania*), and one unidentified species. That this was not a random sample of the insects flying near the spiders was shown by placing sticky traps designed to mimic spider webs (*10*) near the spiders; they captured a large number (N = 145 on 17 trap nights) of (in decreasing order of abundance) flies, beetles, Hemiptera, Homoptera, and other insects, but only one moth of the species caught by the spiders. Collections in the adjacent field (*11*) yielded 71 species of moth in at least eight families, including 36 in the family Noctuidae, confirming the specificity of the spiders' attraction.

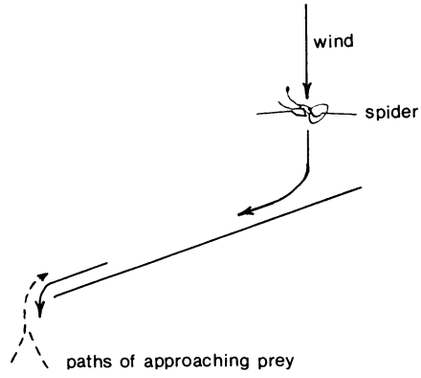

Fig. 2. Deflection of approaching prey with a windscreen.

Spodoptera frugiperda females are known to produce a pheromone that attracts conspecific males (*12*), and it thus seems probable that the spiders use a chemical attractant that mimics this substance. Sex attractant pheromones are widespread in the family Noctuidae, and attraction of more than one species to a given substance is known (*13*). Since individual spiders caught different moth species, they may differ with respect to the attractant they use. *Spodoptera frugiperda* is a pest of sugar cane and other crops (*14*), and it is conceivable that the spiders could be useful in its control.

This unusual hunting technique appears to be approximately as effective for prey capture as building orb webs. Spiders attracted an average of about one moth every 6 minutes when in a hunting position (*15*), and although most moths did not come close enough to be attacked and the spiders missed others (*16*), the spiders averaged at least 2.2 captures per night of hunting (*17*). This converts to about 18 percent of the spider's body weight captured per day (*18*), and is the same as the predation rate of a conventional orb weaver of about the same size, namely, *Argiope argentata* (*19*). The absolute efficiency of the technique is uncertain, however. Bolas spiders almost certainly invest less in webs than do conventional orb weavers, but the metabolic costs of the production of attractive substance and the sometimes prolonged alert waiting periods before prey is captured are unknown, so the relation between gain and loss cannot be determined.

The bolas technique presents an interesting problem. Newly emerged *Mastophora* sp. weigh only about 0.4 mg, less than 1 percent of the weight of the moths attracted by adult females. Whether they are able to attract other smaller prey using different substances, or whether they use some other trapping technique is unknown.

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3. W. Gertsch examined the spider and determined that the species is new; a description of it is being prepared, and specimens will be deposited in the Museum of Comparative Zoology, Cambridge, Mass. 02138.
4. The study was done from January to March, and during July 1977 on the Melendez campus of the Universidad del Valle, Cali, Colombia, and included more than 25 hours of direct observation at night. The campus is in the midst of extensive sugar cane fields. The spiders, all mature females, were in a 30-m stretch of a barbed wire fence (3 m high) in the middle of a large (about 100 by 150 m) field of grass and weeds. Their behavior and that of their prey was observed both by the light of several street lights about 70 m away, and with a headlamp. The headlamp had no appreciable effect on the behavior of the prey, and typically was lit only after prey had approached the spider. Winds during observation periods were light (0 to 6 km/hour); they were most frequently from the south, but changed erratically to other points of the compass.
5. The ball was collected with a minimum of disturbance by cutting it loose as it swung free the moment the spider finished producing it. In such cases the spider usually walked back and forth several times on the horizontal line before settling down in its attack posture.
6. A ball placed on a piece of filter paper which was then placed in a screen cage (0.7 by 0.7 by 0.7 m) containing six male *S. frugiperda* moths (captured less than 2 hours before as they hovered near a spider) also failed to elicit any response. Balls are eaten about every 30 minutes and then replaced, the probable reason for this being that the ball loses its stickiness as it dries out.
7. The exception was a spider from which I had taken a ball; after assuming a predatory stance for about 15 minutes, she resumed the resting position, but a moth hovered nearby off and on for the next 3 minutes.
8. Horizontal sticky traps were placed about 50 cm below the spiders to collect discarded prey, and checked daily. Prey items were not greatly damaged, but were easily distinguished from other insects by their silk wrapping.
9. Dr. E. L. Todd kindly identified specimens of the moths. It was not possible to determine the *Leucania* to species, and more than one species may have been present. Specimens have been deposited in the U.S. National Museum, Washington, D.C. 20560, and the Departamento de Biología, Universidad del Valle, Cali, Colombia.
10. The traps consisted of arrays of sticky nylon monofilament supported by aluminum frames (23 by 32 cm). (W. G. Eberhard, *Bull. Br. Arach. Soc.*, in press). They were hung in the fence at the same height as the spiders.
11. Moths were collected in the adjacent field both by spotting resting individuals with a headlamp (about 10 hours on four nights) and at a light trap (6 hours on two nights). The numbers given are certainly underestimates of the numbers of species actually present. Specimens are deposited in the Departamento de Biología, Universidad del Valle, Cali, Colombia.
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15. I noted 86 moth approaches in 517 minutes on 12 different nights. A moth was scored as having approached if it hovered for more than 3 seconds within 1 m downwind of a spider.
16. Of 82 approaches, 20 resulted in strikes by the spider, and 40 percent of these strikes were successful.
17. After 54 spider nights, 121 discarded moths were found in the traps under the spiders. These counts are conservative since winds and spider movements probably caused some discarded prey to fall free of the traps. Spiders took about 60 to 90 minutes to consume each moth caught, and did not hunt as they fed.
18. Moths averaged 60 mg (wet weight) ($N = 9$). The spiders' weights fluctuated as a result of periodic production of egg masses weighing about 350 mg; they probably averaged about 750 mg.
19. M. Robinson and B. Robinson, *Zool. J. Linn. Soc.* **49** (No. 4), 345 (1970); mature female *A. argentata* weigh about 500 mg.
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Characterization of Bacterial Growth by Means of Flow Microfluorometry

Abstract. *By means of flow microfluorometry, the protein and nucleic acid contents of individual bacterial cells may be measured at the rate of several thousand cells per second. Accumulation of such information over a few minutes yields the composition distribution of the microbial population. These distributions have been determined at different times during batch growth of* Bacillus subtilis, *and the results indicate that the variance of cell composition decreases as the population passes through the exponential into the stationary phase. The relative abundance of endospores and vegetative cells as well as the protein distributions of these subpopulations may be readily determined from flow microfluorometry data. Experimental access to such details of microbial population dynamics should foster improved understanding of cell growth, spore germination, and spore formation kinetics.*

Experimental methods for observing and characterizing a population of growing microorganisms may be divided into two broad categories: those which determine properties averaged over a large number of cells and those which measure individual cell characteristics. Methods such as turbidimetry and conventional biochemical analyses belong to the first class, and have the advantage of providing data on relatively large-sized samples. However, these techniques by definition provide no information on differences in size and composition among individual cells in the population.

Unless special methods are used to obtain synchrony, a pure bacterial culture is a heterogeneous population in which young cells and old cells, single cells and cell chains, and vegetative cells and endospores influence each other through their interaction with a common environment. To understand these interactions one needs information on the distributions of various classes of the cell population under different growth conditions. Statistically significant measurements of these distributions for classification according to size have been obtained according to the Coulter principle for a number of bacteria, including *Escherichia coli*, *Azotobacter agilis*, and *A. vinelandii* (*1*).

The laser flow microfluorometer (FMF) (*2*), which has been applied almost exclusively to mammalian cells in the past (*3*), provides a convenient means of rapidly measuring the protein and nucelic acid content of individual cells. In this instrument cells stained with specific fluorescent dyes flow at rates of 500 to 3000 cells per second through a 0.5-watt continuous argon laser (488 nm). The resulting fluorescence, which is proportional to cellular content of the stained component, is detected by photomultiplier tubes for storage in a computer or multichannel pulse height analyzer.

We have used the FMF to study changes, during batch growth, in the composition distributions of *Bacillus subtilis* ATCC 6051a. The organism was grown at 34°C in a semisynthetic medium described by Jensen (*4*), modified by using 2 percent (weight to volume) glucose instead of maltose. Previously adapted midstationary cells were inoculated into 100 ml of medium contained in 250-ml Erlenmeyer flasks and placed on a rotary action shaker at 300 rev/min. Growth was monitored by means of a Klett-Summerson colorimeter (blue filter) (see Fig. 1, inset). The lag phase observed under our culture conditions lasted about 3 hours, and subsequent exponential growth ceased after approximately 4 hours. At intervals over a 9-hour period, samples of the cell population were harvested, fixed in 70 percent ethanol, and stained with either fluorescein isothiocyanate (FITC, specific for protein) or propidium iodide (PI, specific for double-stranded nucleic acid) (*5*).

Figure 1 illustrates the changes in the population's nucleic acid distribution which occur during batch growth. The average cellular nucleic acid content, which is proportional to the first moment of these distributions, decreases as the bacterial population moves from the early exponential phase (Fig. 1A) to the exponential-stationary transition (Fig. 1B) and finally to the stationary (Fig. 1C) phase of growth. These trends are con-