adult females lacked such spines, possessing instead a single claw.) The shape, relative size, and number of spines varied among the species (*14*); however, their distribution usually was along the anterior-posterior axis within a roughly elliptical boundary when the tarsus was viewed apically (Fig. 1I). These spines probably serve as holdfasts by catching onto irregularities in the substratum and thus prevent or reduce slipping of the palp during oscillations of the tibio-tarsal joint. Their anterior-posterior distribution supports this idea. During attempted sound production on glass surfaces the male's palps repeatedly slide posteriorly. Temporary attachment of the palp may be essential for obtaining maximum pressure by the tarsal scraper against the tibial file.

By coupling the tarsus to the substratum, the spider increases the communicatory effectiveness of the stridulatory apparatus since solid-borne vibrations are conducted directly into the substratum (*15*). Playback experiments suggest that female wolf spiders orient better to substratum vibrations than to airborne sounds (*4*). Coupling may incorporate the substratum into the system as a sounding-board, thereby increasing the loudness of the airborne component. The airborne sound probably plays a role (for example, reduces the prey-capture tendency of the female) prior to the time that both spiders are located on the same substratum during the final phase of precopulatory display. Both the loudness and the regularity of the fine structure of the sounds produced by male lycosids such as *L. gulosa* (*2*) can be understood on the basis of this substratum-coupled stridulatory device. Clearly, "percussion" no longer denotes correctly the primary mechanism used by most sound-producing male wolf spiders (*16*).

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7. After being examined under a dissecting microscope, some of the material was coated with gold (without prior dehydration) and immediately examined with an Hitachi HSS 2R scanning electron microscope.
8. The stridulatory organ components were largest relative to the size of the palp in *S. crassipes* and *S. saltatrix*. They were relatively smaller but still well developed in *L. aspersa, L. carolinensis, L. gulosa, L. punctulata, L. rabida*, and *S. avida*. The small, narrow file in *L. Helluo* was rather difficult to detect. Sounds have been recorded from males of all these species [(*2, 4, 5*); also J. S. Rovner, unpublished data].
9. Although lacking morphological adaptations for sound production, adult female *Lycosa* spp. produce faint sounds by scraping the tarsal claw against the substratum during agonistic displays (J. S. Rovner, unpublished data).
10. R. Legendre, *Ann. Biol.* **2**, 371 (1963). The stridulatory devices described previously in spiders involved three basic forms: the abdomen rubbing against the prosoma or pedicel (types a and b), one appendage rubbing against another (types c to f), or one appendage rubbing against the abdomen (type g). The only stridulatory organ heretofore reported in a lycosid was of type g and occurred in *Pardosa fulvipes* (Palearctic) [T. Kronestedt, *Zool. Scripta* **2**, 43 (1973)].
11. Two *L. rabida*, two *S. crassipes*, and one *S. saltatrix*. Placing both palps in a human hair sling with its ends attached to the middle of the carapace prevented the spiders from "chewing" off the paraffin casts prior to testing, at which time the palps were freed from the sling. This precaution was necessary only for *L. rabida*; the others courted soon after the casts were applied.
12. Solid-borne sounds were detected with a high-sensitivity vibration pickup system (type 1560-P14, General Radio, West Concord, Mass. 01781) connected to a sound-level meter (type 1551-C, General Radio), whose output was fed into a tape recorder. The same paper substratum was used during all recordings.
13. When all palpal movement is prevented (by attaching the palps to the prosoma with paraffin), a very faint "whirring" sound still occurs during display in *L. rabida*. It apparently arises from the simultaneous vibrations of the forelegs and abdomen. The intensity of this sound was reduced but not eliminated after attachment of the abdomen to the prosoma by a paraffin bridge.
14. The presence of such spines in illustrations of male palps in taxonomic reports treating various species of *Lycosa* and *Schizocosa* (including other than Nearctic forms) suggests that the sound-producing mechanism described here may be found in most (or all) species in these genera.
15. This would be most effective if it is the file that is setting the tarsal scraper into vibration, as could be the case [B. Dumortier in *Acoustic Behaviour of Animals*, R. G. Busnel, Ed. (Elsevier, Amsterdam, 1963), p. 279)].
16. A few male lycosids do rely on percussion. According to O. von Helversen (personal communication) the male *Hygrolycosa rubrofasciata* (Palearctic) taps dry leaves with its abdomen, whose ventral surface has a sclerotized plate for this purpose. Male *Alopecosa pulverulenta* (Palearctic) [Bristowe and Locket (*3*)] and *A. aculeata* (Nearctic) (*5*) also are reported to use abdominal and palpal percussion as a mode of sound production.
17. Supported in part by NSF grant BMS-7101589. I thank P. L. Rovner for valuable discussions, R. D. Gaarden for operating the scanning electron microscope, and J. A. Wilson for reading the manuscript.

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Lymphocytes of the Toad *Xenopus laevis* Have the Gene Set for Promoting Tadpole Development

Abstract. *Nuclear transplantation experiments show that differentiated cells, such as lymphocytes, from the adult frog can express the genes necessary for tadpole development. The transplanted cells were proven to be lymphocytes by immunological methods. The origin of the tadpoles that developed after lymphocyte nuclei injections was ascertained by a karyotypic marker.*

A better knowledge of the genetic content of a "pre-committed" lymphocyte is needed for understanding the mechanism of antibody formation. Moreover, the lymphocyte can be used for investigating whether such differentiated cells have the full gene set needed for promoting development of normal individuals in nuclear transplant experiments.

Nuclei of embryonic amphibian cells can give rise to normal frogs when injected into enucleated amphibian eggs (*1*). Several reports suggest that development could occur after nuclear transplantation of differentiated normal cell nuclei (*2*). Convincing evidence has been provided by the experiments of Gurdon *et al.* (*3*) where both the differentiation state of the cells that were injected into enucleated eggs and the genetic origin of the developing embryos were established. Here, we report on the development after transplantation of adult *Xenopus laevis* lymphocyte nuclei.

Table 1. Genetic origin of the clones of serial transfers, as followed by the nucleolar marker. One to four individuals per clone were checked. Chromosome numbers were derived from 6 to 20 metaphases per individual; nucleolus numbers were ascertained from squash preparations under the phase contrast microscope.

Number of clones	Number of chromosomes	Number of nucleoli	Origin of the clones
6	36	1	Lymphocyte
2	~72	3	Unclear
1	32	2	Unclear

Individuals of *Xenopus laevis* one nucleolar (1-nu) mutant (*4*) were immunized by one injection of 2,4-dinitrophenyl (DNP)-hemocyanin from the keyhole limpet. The spleens of the *Xenopus* were removed 7 to 15 days later and teased in Wolf and Quimby culture medium; the resultant cell suspensions were washed twice in the same medium. The spleen cells obtained in this way were then placed on nylon grids to which DNP had been coupled according to the Kiefer modification (*5*) of a method of Edelman *et al.* (*6*). To show that the cells coupled to the grid were differentiated immunoglobulin-bearing cells, we assayed the inhibition of binding after treatment with antiserum to immunoglobulin. The lymphocyte population was treated with antiserum to immunoglobulin of defined specificity (*7*); the lymphocytes were then exposed to the grid. Under these conditions, only 49 cells bound to the grid, although we counted 2390 binding cells in the control preparation (pooled data of

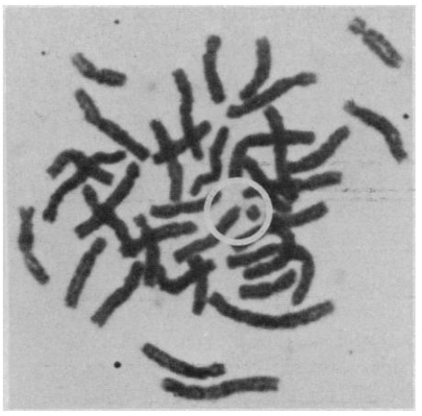

Fig. 1. Karyotype of a one-nucleolar diploid individual resulting from nuclear transplantation of a lymphocyte. There is the diploid set of 36 chromosomes but only one chromosome bears a functional nucleolar organizer (circle). The chromosome in the upper right corner was out of field for high magnification photography, and was inserted afterward.

three assays). Thus, 98 percent (96.1 to 98.7 percent at 99 percent confidence limit) were immunoglobulin-bearing and therefore were considered to be lymphocytes.

The antibody to 2,4-DNP specificity of the lymphocytes was ascertained by inhibition experiments: the cells in suspension were reacted with the 2,4-DNP grids in the presence of different concentrations of DNP-lysine as inhibitor. Concentrations such as $3 \times 10^{-4}M$ or $6 \times 10^{-4}M$ of 2,4-DNP-lysine inhibited the binding by 75 to 85 percent, whereas the cross-reacting ligand 2,6-DNP-lysine (*8*) inhibited only 40 to 70 percent.

The grids were transferred to calcium- and magnesium-free isotonic phosphate-buffered saline. The bound cells were sucked into a micropipette and immediately injected into enucleated wild-type eggs. Nearly 100 percent of the cells taken from the grid in this way had ruptured cell membranes, as indicated by trypan blue uptake (*6*). The disruption of the cell membrane is necessary for successful development of somatic nuclei in nuclear transplantation experiments (*9, 10*). To kill the egg pronuclei the eggs were treated with ultraviolet radiation (*11*). In addition, the egg nuclei were drawn out by a micropipette to increase the number of successful enucleations, since in our experience the ultraviolet irradiation was not as efficient as reported.

Of 100 injected eggs, 20 underwent cleavage; 12 developed into complete blastulas, nine of them into gastrulas, two of which were not 1-nu. Three of the 1-nu gastrulas looked almost normal. The blastulas and gastrulas were disrupted, and their cells were used in serial transfers (*12*) (nine donors giving nine clones). Thus the nuclei of these cells were again injected into enucleated eggs, in order to allow the lymphocyte nucleus to express its full developmental capacity, which could be impaired at the first transfer for technical reasons, or because of division asynchrony of the egg and the injected nucleus.

Nucleolar counts and karyotypes were made of embryos (usually tailbud stage, muscular response) obtained in each of these nine clones. In six clones, the number of chromosomes was 36 (diploid) (Table 1). In chromosome preparations, only one chromosome bearing the nucleolar organizer was identified (Fig. 1). The tadpoles of one of the 1-nu diploid clones were normal (Fig. 2) up to stage 43 to 44 [described by Nieuwkoop and Faber (*13*)]; at this stage the tadpoles developed edema and died. In the other cases the swimming tadpoles developed their abnormalities earlier. Abnormalities of embryos may also occur when blastula nuclei are transplanted. Therefore it does not necessarily indicate that the transplanted cells were missing some of the genetic material of the species.

To interpret nuclear transplantation experiments, it is necessary to check both the differentiation state of the cells whose nuclei were transplanted and the origin of the resulting embryos. That 75 to 85 percent of the cells are inhibited by DNP-lysine is not an indication that only this percentage of cells carry immunoglobulins specific for DNP. On the nylon grid, DNP acts as a multivalent antigen, thus competing more efficiently than the free hapten for the surface immunoglobulins. Conversely, passively acquired immunoglobulins (cytophilic antibodies) could decrease the percentage of lymphocytes that synthesize immunoglobulins with DNP specificity.

In any case, 98 percent of the bound cells were immunoglobulin-bearing cells. In the mouse, 97 percent of the cells bound were killed when the T (thymus-derived) and B (bone marrow–derived) cells were treated with antiserum to theta and immunoglobulin (*14*). In *Xenopus laevis*, both T and B cells carry immunoglobulins (*15*). Therefore both populations can be inhibited by treatment with antiserum to immunoglobulin. The same treatment would not inhibit the binding of macrophages (*16*), and therefore these cells are not included in the 98 percent population of cells discussed above. Most of the macrophages adhered to the glass vials during the preparation procedure, as evidenced by microscopic observations of the glass surfaces. The few that could have stuck to the nylon were completely flattened on the fiber, and therefore hardly visible. If they were visible, they would have been included into the 2 percent of cells that were not proven lymphocytes. Moreover,

Fig. 2. The "most-normal" tadpole we obtained from a lymphocyte. This tadpole belongs to the same clone as the individual giving the karyotype shown in Fig. 1.

they could not be mistaken for lymphocytes, which stick out of the nylon fiber. The probability that this 2 percent of cells could have developed into six gastrulas, and further into six clones, was examined statistically. Using the Poisson approximation to the binomial distribution we found that the probability that this could be the case is $P < .01$, under the unrealistic assumption that every injected cell with full genetic potential gives rise to a gastrula. The transplantation yield of the most undifferentiated cells, the blastula cells, in our experiment was 25 percent. Many reasons for that fact have been suggested (*10, 17*). Taking that into account, we found that the probability that all six clones were derived from the 2 percent population which were not proven lymphocytes is $P = .00001$. Therefore, we conclude that nearly all animals that developed after transplantation were lymphocyte derived.

Since the cells were withdrawn during the proliferation phase, lymphoblasts were among the transplanted cells. According to Briggs and King (*17*) this could be one reason for the success of transplantation.

The determination of the origin of the embryo is needed to discriminate the gynogenetic animals from the lymphocyte-derived animals. Certitude that the animals are lymphocyte derived is only possible when the karyotype of the 1-nu embryo shows 36 chromosomes, among which one bears the secondary constriction of the nucleolar organizer. In fact, when the ultraviolet irradiation of the recipient eggs was not combined with surgical removal of the egg pronucleus, gynogenetic development was frequent, leading in some cases to normal diploid 2-nu tadpoles that lived for more than 2 months. One even metamorphosed. Beside 1-nu haploids, triploids, and individuals with polynucleolated cells, apparently 1-nu embryos that were aneuploid (near diploid) were also scored. In that case the estimation of the nucleolar number could have been misleading because of eventual loss or duplication of the chromosomes bearing the nucleolar organizer. Altogether we recorded 30 gynogenetic tailbud stage embryos or tadpoles derived from 1500 injected eggs. The incidence of such gynogenetic animals was decreased, but not eliminated, when

the double enucleation technique was used.

So far, there does not exist a normal adult frog unequivocally derived from the transplantation of the nucleus of a differentiated cell. In the experiments of Gurdon *et al.* (*3*) the ultimate stage of development was similar to the one we recorded.

With the provision that we do not know the number of genes necessary to code for an animal developed as far as described, we conclude that differentiated cells such as lymphocytes from adult frogs can express the set of genes necessary for tadpole development.

For immunologists, tadpoles old enough to be assayed for their immunological responsiveness could provide a way to examine the genetic potential of a single lymphocyte. Since any somatic changes will be "frozen" in the newly arisen animals, we believe that such lymphocyte-derived tadpoles may be a useful model to approach the problem of antibody diversity, as well as the phenomenon of allelic exclusion and the possible occurrence of somatic translocation or recombination in the genetic regions coding for immunoglobulins.

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Acoustically Orienting Parasitoids: Fly Phonotaxis to Cricket Song

Abstract. *Larviparous female tachinid flies are attracted to taped cricket songs. In the laboratory flies deposit larvae on a cricket mounted to a speaker; the larvae burrow through the cricket's exoskeleton and develop internally. These acoustically orienting parasitoids probably influence male reproductive behavior and sexual competition in crickets.*

Although it has long been considered possible that some predators could locate prey by using the acoustical signals of the prey, Walker (*1*), working with domestic cats, has provided what is, to my knowledge, the only demonstration that a predator can orient acoustically to singing prey. I here report a parasite that locates a host by using the song of the host.

During the summers of 1974 and 1975 I observed that a tachinid fly, *Euphasiopteryx ochracea*, was attracted to the tape-recorded song (*2*) of the field cricket *Gryllus integer*. I collected 11 flies on two nights in 1974 and 87 flies during a 14-night period in 1975 (*3*). Dissection of 35 flies showed that all were females and that each contained living larvae.

To observe fly behavior more closely and to demonstrate the phonotactic response of the flies, I released living flies one at a time into a box measuring 0.8 by 0.8 by 1.2 m. The box was lined with acoustical tile, and I observed the flies through a window in the box. On the floor of the box were two speakers, each with a dead cricket attached (*4*). Cricket songs were played over one speaker while various control sounds (*5*) were played over the other. Both types of sound were produced at the

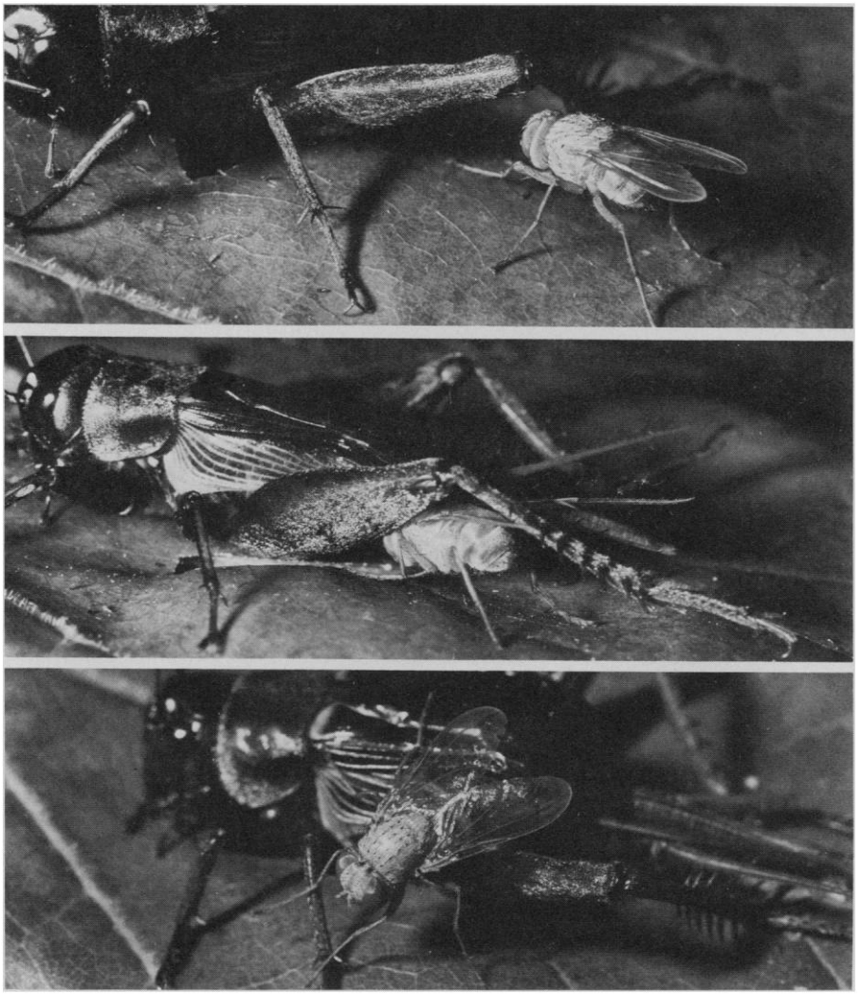

Fig. 1. Views of a fly (*Euphasiopteryx ochracea*) approaching and attacking a dead cricket (*Gryllus integer*) mounted on the top of a speaker that is producing cricket song. This fly measured 7 mm in length.