collagenase inhibitory activity suggests a possible physiological role for this protein in connective tissue metabolism. Platelets are normally activated during in vivo clot formation or tissue injury. Such injury induces an inflammatory reaction with attraction of granulocytes to the injured area. The precise nature of the interactions among platelets, collagen, released PF4, granulocytes, and collagenase remains to be elucidated.

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References and Notes

1. S. Niewiarowski and D. P. Thomas, *Nature (London)* **222**, 1269 (1969); A. Youssef and P. Barkhan, *Br. Med. J.* **1**, 746 (1968).
2. S. P. Levine, H. Wohl, U. Marzec, E. F. Bernstein, J. Kroener, *Thromb. Res.* **10**, 1 (1977).
3. S. P. Levine and H. Wohl, *J. Biol. Chem.* **251**, 324 (1976).
4. T. F. Deuel, P. S. Keim, M. Farmer, R. L. Heinrikson, *Proc. Natl. Acad. Sci. U.S.A.* **74**, 2256 (1977).
5. E. F. Lüscher and R. Käser-Glanzmann, *Thromb. Diath. Haemorrh.* **33**, 66 (1975).
6. S. Seifter and E. Harper, in *The Enzymes*, P. D. Boyer, Ed. (Academic Press, New York, 1971), vol. 3, p. 649.
7. G. S. Lazarus, J. R. Daniels, R. S. Brown, H. A. Bladen, H. M. Fullmer, *J. Clin. Invest.* **47**, 2622 (1968).
8. K. E. Kuettner, J. Hiti, R. Eisenstein, E. Harper, *Biochem. Biophys. Res. Commun.* **72**, 40 (1976).
9. K. E. Kuettner, L. Soble, R. L. Croxen, B. Marczynska, J. Hiti, E. Harper, *Science* **196**, 653 (1977).
10. A. Z. Eisen, J. J. Jeffrey, J. Gross, *Biochim. Biophys. Acta* **151**, 637 (1968).
11. J. Mendelsohn, A. Skinner, S. Kornfeld, *J. Clin. Invest.* **50**, 818 (1971).
12. W. A. Skoog and W. S. Beck, *Blood* **11**, 436 (1956).
13. G. S. Lazarus, R. S. Brown, J. R. Daniels, H. M. Fullmer, *Science* **159**, 1483 (1968).
14. Y. Nagai, C. M. Lapiere, J. Gross, *Biochemistry* **5**, 3123 (1966).
15. Performed by Dr. H. Ginsburg.
16. Experiments conducted by Dr. B. Österud.
17. A. E. Bolton, C. A. Ludlam, D. S. Pepper, S. Moore, J. D. Cash, *Thromb. Res.* **8**, 51 (1976); S. P. Levine and L. S. Krentz, *ibid.* **11**, 673 (1977).
18. O. Warburg and W. Christian, *Biochem. Z.* **310**, 384 (1941).
19. O. H. Lowry, N. J. Rosebrough, A. L. Farr, R. J. Randall, *J. Biol. Chem.* **193**, 265 (1951).
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Temperature Coupling in the Vocal Communication System of the Gray Tree Frog, *Hyla versicolor*

Abstract. *The gray tree frog mates over a temperature range of at least 9°C. Gravid females, tested at two different temperatures, preferred synthetic mating calls with temperature-dependent temporal properties similar to those produced by a male at about the same temperature as their own. Thus, the vocalization system and the temporal pattern recognition system are affected by temperature in a qualitatively similar fashion.*

Several properties of the mating calls of the gray tree frog, *Hyla versicolor*, are temperature-dependent (*1*). Since these frogs call over a temperature range of at least 13°C (*2*), the question of whether females respond differentially to vocalizations produced over this temperature range arises (*3*). In my experiments, females discriminated between synthetic mating calls on the basis of temperature-dependent temporal properties; furthermore, the females preferred calls with temporal properties similar to those produced by a conspecific male at about the same body temperature as their own. Despite the widespread implicit assumption that this phenomenon, which I term temperature coupling, occurs in the sound communication systems of poikilothermic animals (*4, 5*) my experiments are, to my knowledge, the first demonstration in vertebrates and the first corroboration of the classic study of crickets published more than 20 years ago by Walker (*6*).

Synthetic mating calls were generated by summing two phase-locked sinusoids of 1.1 kHz (−6 dB) and 2.2 kHz (0 dB), modulating this waveform to produce pulses of variable pulse-repetition rate (PR) and duration (PD), and then superimposing another stage of modulation on trains of such pulses to produce calls. The PR's were chosen on the basis of a linear regression analysis of the mating calls of 39 males with body temperatures between 12.8° and 26°C (Fig. 1) (*7*). Further analysis indicated that PD was inversely correlated with temperature so that the duty cycle (PD divided by pulse period) tended to remain constant. [Although there was a slight tendency for the number of pulses per call (PN) to decrease with rising temperature, PN was variable within and among individuals; therefore I decided to hold PN (14 pulses) as well as the duty cycle ($\sim$0.6) constant in the two principal experimental stimuli (Fig. 2. A and B).] I used three additional experimental stimuli: a typical natural call (Fig. 2C), recorded at 23°C, and two synthetic versions. One synthetic call (Fig. 2D) was composed of frequency-modulated (FM) pulses, and the other (Fig. 2E) lacked FM pulses (*8*). Each kind of call was recorded on magnetic tape on one channel of a stereophonic recorder (Nagra) and had a fixed timing relationship to another call on the other channel (Fig. 2A) (*9*).

Discrimination experiments took place in a dimly lit semianechoic, temperature-regulated (±1°C) chamber (4 m by 2 m by 1.8 m). A stereophonic recorder (Nagra) drove two speakers (Nagra) placed 2 m apart on a plywood floor. Sound pressure levels (SPL's) of the two sounds were equalized midway between the speakers (*10*). Twenty-six amplexed pairs were collected in Boone County, Missouri, in early May 1977. Each gravid female was separated from the male and either refrigerated at 4°C (to inhibit oviposition) for as long as 18 hours before testing or placed directly into the chamber. After a minimum of 20 minutes of acclimation in the chamber, each female was placed in a petri dish midway between the speakers and restrained with a hardware cloth cage until both sounds had been played several times. After the female was freed, a response was tabulated if she touched a speaker or came within 5 cm of it. The cloacal temperature of the animal was taken immediately with a quick-reading thermometer (Schultheis).

Eighteen females responded in at least one discrimination experiment. Twelve females, tested at 75 dB, chose the synthetic call with a PR of 15 sec^{-1} when their body temperatures were about 16°C. Ten females, tested at 75 dB, chose the synthetic call with a PR of 24 sec^{-1} and one chose the synthetic call of 15 sec^{-1} when their body temperatures were about 24°C (Table 1). These responses were obtained from a total of 14 females. Six that first responded at 24°C later responded at 16°C; three that first responded at 16°C later responded at 24°C. The one female that chose the 15 sec^{-1} call at 24°C initially chose the 15 sec^{-1} at 16°C. At least 6 hours elapsed between the first test at 75 dB at one temperature and the second test at 75 dB at the other temperature. (Females were refrigerated at 4°C until 20 minutes before the second test.) Clearly, the females preferred the calls with temperature-dependent temporal properties similar to those produced by a conspecific male at about the same temperature as their own.

Tests at 85 dB were conducted within a few minutes of the 75-dB test. Each female was replaced at the central release point, and the stimuli were usually ex-

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changed between the speakers to avoid any possible directional bias. The responses of the 14 females (initially tested at 75 dB) plus the responses of 4 others are given in Table 1. Five of these 18 animals responded at both temperatures. At 16°C, the females chose the 15 sec^{-1} call; at 24°C, they failed to discriminate (*11*). At 24°C, the females typically oriented and moved first toward one speaker and then toward the other as the sounds were alternately broadcast. With one or two exceptions, other responses at 24°C (75 dB) and at 16°C (75 dB and 85 dB) were much more deliberate.

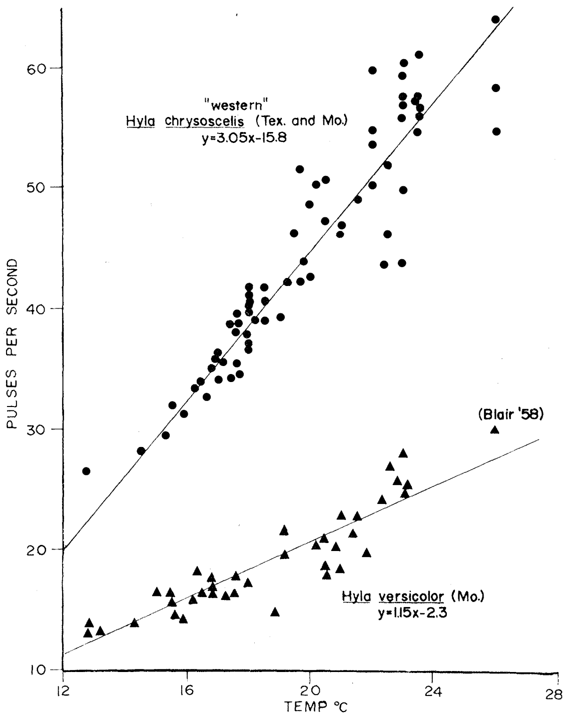
Ten animals were tested (at 75 dB) in the control experiments within a few minutes of the test at 85 dB and 24°C (*12*). The synthetic FM call was as attractive as the natural call, and the synthetic call without FM pulses was as attractive as the call with FM pulses (Table 1C). These results indicate that the principal synthetic signals were behaviorally equivalent to natural mating calls despite the absence of FM pulses. Since the two principal calls had the same spectral properties, temperature coupling was based on differences in temporal properties.

In widespread localities throughout the eastern half of the United States, *H. versicolor* breeds in the same ponds with its sibling species, *Hyla chrysoscelis*. Since the latter species is a diploid and *H. versicolor* is a tetraploid, matings between the two result in the production of sterile, triploid hybrids (*13*). The calls of *H. chrysoscelis* also consist of pulse trains in which PR and PD vary with temperature (Fig. 1) (*14*). The PR's of *H. chrysoscelis* calls are considerably higher than those of *H. versicolor* at the same temperature, but at low temperatures (<17°C), *H. chrysoscelis* produces calls with PR's similar to those of *H. versicolor* at high temperatures (>23°C). At the low temperature (16°C), females of *H. versicolor* responded exclusively to the synthetic call with a lower PR at both 75

Table 1. Responses of 18 female *Hyla versicolor* in discrimination experiments.

Test temperature (°C)	Type of call	Number of females responding	Type of call	Number of females responding
		Responses at 75 dB SPL†*		
16‡	Synthetic with PR = 15 sec^{-1}	12	Synthetic with PR = 24 sec^{-1}	0
24§		1		10
		Responses at 85 dB SPL*		
16	Synthetic with PR = 15 sec^{-1}	8	Synthetic with PR = 24 sec^{-1}	0
24		9		6
		Control experiments at 75 dB SPL		
24	Natural (Fig. 2C)	4	Synthetic with FM (Fig. 2D)	6
24	Synthetic with FM (Fig. 2D)	5	Synthetic without FM (Fig. 2E)	5

*For some females, two responses were tabulated, each at a different temperature. †Re 2×10^{-4} μbar root mean square. ‡Cloacal temperatures of the females ranged from 15.2° to 16.8°C, with one exception (14.8°C). §Cloacal temperatures ranged from 23.6° to 25.2°C, with one exception (26°C).



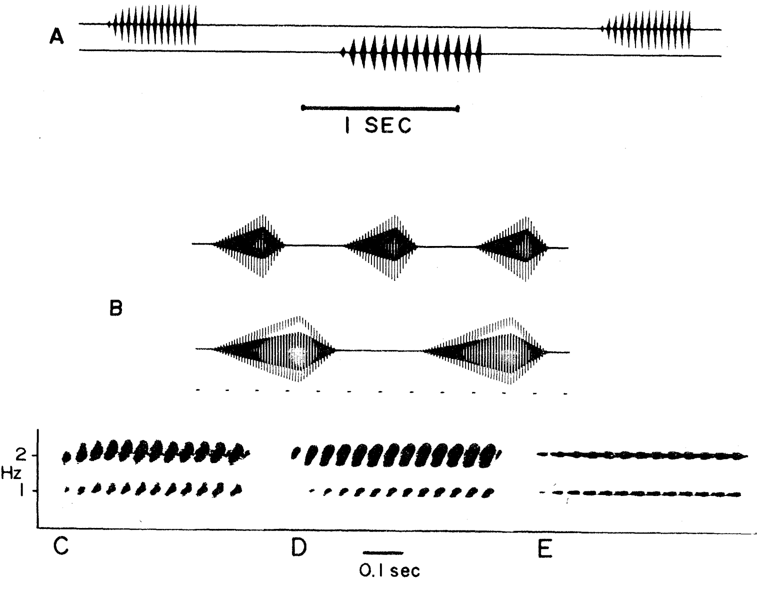


Fig. 1 (left). Scatter diagram and fitted linear least-squares regression lines of pulse-repetition rates of the mating calls of *H. versicolor* and *H. chrysoscelis* versus temperature. One point for *H. versicolor* (Blair) was taken from the literature (*Blair '58*) (*1*); the male was recorded in Texas. The other males of *H. versicolor* were recorded in Missouri. There were no significant differences in the PR's (after correction for temperature) or the slopes of the linear regression lines between a sample of 24 males of "western" (*14*) *H. chrysoscelis* from Missouri and a sample of 45 *H. chrysoscelis* from Texas supplied by D. B. Ralin. These data have thus been combined and labeled "western *Hyla chrysoscelis*." Notice that the pulse rates of "cold" *H. chrysoscelis* are similar to those of "hot" *H. versicolor*. Fig. 2 (right). (A) Oscillograms of the two principal synthetic calls, showing their timing relationship during presentation to females of *H. versicolor*. The call with 24 pulses per second (upper trace) was played from one speaker and corresponds to a natural call recorded at about 24°C. The call with 15 pulses per second (lower trace), which corresponds to a natural call recorded at about 16°C, was played from another speaker located 2 m away. The first synthetic call that a female heard was determined in an unbiased fashion. (B) Oscillograms with an expanded time base (time markers are 10 msec apart) of representative pulses of the two principal synthetic calls. The call with 24 pulses per second is shown in the upper trace, and the call with 15 pulses per second, in the lower. (C) Sonagram (narrow band) of a natural call of a *H. versicolor* recorded at 23°C. This call was used in one of the control experiments in which it competed with the synthetic call illustrated by a sonagram in (D). Both of these sounds have frequency-modulated pulses. (E) Sonagram (narrow band) of a synthetic call lacking FM pulses, as do the two principal synthetic calls.

and 85 dB (Table 1). At 24°C, females failed to discriminate between calls with PR's of 15 sec^{-1} and 24 sec^{-1} at 85 dB (Table 1), but the PR's of *H. chrysoscelis* calls at 24°C are much higher than those of *H. versicolor*, even at 26°C (Fig. 1). It will be important to test females at 24°C and 85 dB for their ability to discriminate between a call with a PR of 24 sec^{-1} and a call with a higher PR, in the range of variation of *H. chrysoscelis* calls at 24°C. In any event, the biological significance of temperature coupling appears to be the refinement of species identification in order to avoid mismating and gamete wastage (*15*).

The phenomenon of temperature coupling in the gray tree frog provides an exciting opportunity for the neurophysiological analysis of temporal pattern recognition. Spectral properties of the signals are irrelevant, and changes in putative neural correlates of temporal pattern specificity with temperature can be predicted beforehand. Furthermore, the existence of temperature coupling in tree-frog communication is consistent with the hypothesis that the pattern-generating system and the pattern-recognizing system may share the same or similar neural networks as suggested by Hoy (*16*). Alternatively, two independent networks may be affected in a similar fashion. A more detailed analysis of the temperature-coupling phenomenon, especially at temperature extremes where one or both functions may become nonlinear, may help to eliminate one of these hypotheses. Finally, research with electric fish (*17*) and fireflies (*18*), which use signals that have distinctive, temperature-dependent temporal properties, indicates that temperature coupling is not limited to acoustic communication.

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References and Notes

1. W. F. Blair [*Am. Nat.* **92**, 27 (1958)] showed temperature-dependent changes in pulse-repetition rate, call duration, and dominant frequency in the mating calls of *H. versicolor*.
2. I have recorded males between 12.8 and 23.1°C; Blair (*1*) recorded a male at 26°C.
3. I have found females in amplexus at 14° and 23°C.
4. In studies with poikilothermic animals, biologists have often matched carefully the temperature at which they conducted experiments with females to the temperature at which the experimental sounds were recorded. The implicit assumption is that the specificity of the animal's response may be affected by its temperature [(*5*); M. J. Littlejohn, *ibid.*, p. 370; R. Hoy and R. Paul, *Science* **180**, 82 (1973); R. Hoy, J. Hahn, R. Paul, *ibid.* **195**, 82 (1977)].
5. M. J. Littlejohn, M. J. Fouquette, C.Johnson, *Copeia* **1960-II**, 47 (1960).
6. T. J. Walker, *Ann. Entomol. Soc. Am.* **50**, 626 (1957).
7. Analyses of changes in the mating calls of individual males as a function of temperature are consistent with the linear regression analysis.
8. Since PD was changed to keep the duty cycle about the same, either the beginning and ending points of FM or the rate of change of FM would differ if this property were incorporated in the principal experimental calls. Since I wanted to eliminate spectral differences, I gambled that FM was irrelevant and tested this guess in the control experiments.
9. When in close proximity, males of *H. versicolor* tend to avoid calling simultaneously.
10. Sound level meters were General Radio 1933 and Brüel and Kjaer 2209. When the "fast" root-mean-square SPL's of the two calls were equalized, the maximum peak SPL's differed by less than 1 dB. Since the duty cycles of the two calls were about the same, the sound power was about the same. An octave-band analysis at the point where females were released indicated that the spectral compositions of the two sounds were nearly identical.
11. It will be important to learn why females failed to discriminate at 24°C and 85 dB. An SPL of 85 dB is 5 dB *lower* than the mean SPL of the calls of eight males measured in the field at a distance of 1 m [H. C. Gerhardt, *J. Comp. Physiol.* **102**, 1 (1975)]. The physiological state of a female captured in amplexus may influence the selectivity of her responses to sound patterns at higher temperatures as compared with a female in the early stages of responsiveness. Since responsiveness to mating calls is somewhat hormone-dependent [R. S. Schmidt, *Behaviour* **35**, 114 (1969)], this hypothesis can be tested by observing the preferences of gravid females at various times after the injection of exogenous hormones. Another hypothesis is that temperature affects the sensitivity of auditory neurons, as it does in other poikilothermic vertebrates [see, for example, Y. L. Werner, *J. Exp. Zool.* **195**, 319 (1976)], so that saturation (and hence a loss of temporal information) occurs at high but not low temperatures at 85 dB.
12. One female chose synthetic calls with FM and one chose synthetic calls without FM when tested at 16°C. The PR of these calls was 14 per second.
13. A. O. Wasserman, *Science* **167**, 385 (1970); D. B. Ralin, *Copeia* **1976-I**, 191 (1976).
14. Calls of *H. chrysoscelis* also vary geographically in PR [H. C. Gerhardt, *Copeia* **1974-III**, 534 (1974)]. L. Maxson, E. Pepper, and R. Maxson [*Science* **197**, 1012 (1977)] presented immunological evidence that these differences probably reflect species differences and not merely geographical variation. The PR data (Table 1) are from "western" populations of the "fast-trilling" *H. chrysoscelis* found in sympatry with *H. versicolor* in the central United States [J. Bogart, personal communication; see also C. Johnson, *Tex. J. Sci.* **18**, 361 (1966); T. Johnson, *The Amphibians of Missouri* (Natural History Museum, University of Kansas, Lawrence, 1977)].
15. Call discrimination has been demonstrated in *H. versicolor* and *H. chrysoscelis* [(*5*); H. C. Gerhardt, *Behaviour* **49**, 130 (1974); H. C. Gerhardt, *Copeia* **1974-I**, 47 (1974)].
16. R. Hoy, *Am. Zool.* **14**, 1067 (1974).
17. A. S. Feng, *Comp. Biochem. Physiol. A* **55**, 99 (1976).
18. E. Papi, *Monit. Zool. Ital.* **3**, 135 (1969); A. Carlson, J. Copeland, R. Raderman, A. Bulloch, *Anim. Behav.* **24**, 786 (1976).

19. I thank W. Sherman for designing the sound synthesizer; B. Martof, J. Rheinleander, J. Haas, and anonymous reviewers for helpful suggestions about the manuscript; W. Oliver and D. Gayou for field assistance; and B. Witcher for inspiration. This research was supported by NSF grant BNS 73 00795 and research career development award NS00217-021 from the National Institutes of Health.

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Cyclic Polyether–Protonated Organic Amine Binding: Significance in Enzymatic and Ion Transport Processes

Abstract. *The cyclic polyether, 18-crown-6, reacts with protonated amines in methanol to form complexes whose formation constants (log* K*) decrease in the order* NH_4^+, $RNH_3^+ > R_2NH_2^+ > R_3NH^+$. *In the case of the organic amines, this stability order is identical to the earlier observed permeability sequence for protonated organic amines in glyceryl dioleate bilayers treated with valinomycin, nonactin, or gramicidin, and in bullfrog and rabbit gallbladder membranes. The decrease in log* K *values in the above series is primarily a result of decreased enthalpy change (*ΔH*) values, the entropy change (*$T\Delta S$*) term being essentially constant for the systems studied.*

Macrocyclic polyethers of the "crown" type have elicited much interest because of their potential importance in elucidating the mechanism of enzyme action and membrane transport processes. Recently, macrocyclic molecules based on the 18-crown-6 structure [see 1, 4, 7, 10, 13, 16-hexaoxacyclooctadecane (compound **1**)] have been synthesized and shown to display efficient chiral recognition toward various optically active ammonium salts (*1*). Because of their ability to differentiate among inorganic cations and organic stereoisomers, these "functionalized crown ethers" have been suggested as model compounds for the investigation of mechanisms of enzyme action (*2*). Independently, the cyclic antibiotics, valinomycin, nonactin, or gramicidin, have been shown to increase the permeability of glyceryl dioleate bilayers toward protonated organic amines in proportion to the number of protons available for hydrogen bonding (*3*).

This permeability increase is reminiscent of the solubilization of inorganic cations in artificial and natural membranes by cyclic antibiotics and cyclic polyethers (*4, 5*). Likewise, the permeability of bullfrog and rabbit gallbladders membranes toward protonated organic amines depends upon the number of protons available for hydrogen bonding (*3*). These permeability sequences involving organic cations can be formulated as $RNH_3^+ > R_2NH_2^+ > R_3NH^+$. The importance of amine transport in biological systems has long been recognized. Organic amines are known to block either Na^+ or K^+ nerve channels (*3*) and in some cases, result in death to the organism, for example, "red tide" toxin (*6*). Also, amine groups are constituents of many biologically important compounds (amino acids and drugs, for

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