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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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)].
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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)].
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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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example) whose mechanism of transport across membranes is largely unknown.

Earlier, we showed that the aqueous stability sequence based on the formation constant (log K) of dicyclohexo-18-crown-6 toward alkali metal cations was identical to the permeability sequence for this system (*7*). This compound was proposed as a model for the study of active transport processes. Since that time much information has accumulated concerning the selectivities of cyclic polyethers toward inorganic cations (*8, 9*) and in membrane transport (*5*).

We have now determined, using a calorimetric titration procedure described earlier (*9*), log K, enthalpy (ΔH), and entropy ($T\Delta S$) change values valid at 25°C in absolute methanol for the interaction of 18-crown-6 with NH_4^+ and several protonated organic amines as shown in Eq. 1. The stability sequence, based on

$$\text{amine}^+ + \text{18-crown-6} = (\text{18-crown-6-amine})^+ \qquad (1)$$

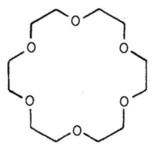
log K values, for these organic amines is identical to the permeability sequence given above. The log K data show the stability of the host-guest complexes to decrease as the number of hydrogen atoms available on the protonated organic amine for hydrogen bonding to the oxygen donor atoms of the cyclic ligand decreases (Table 1). In the case of NH_4^+, only three of the four protons are available for hydrogen bonding to the 18-crown-6. The log K value in the case of NH_4^+ is similar to those for the $CH_3(CH_2)_nNH_3^+$ amines (n = 0, 1, 2), which suggests similar binding in these two cases. This behavior by NH_4^+ is in contrast to the position of NH_4^+ in the above permeability sequence (*3*) where it lies well above RNH_3^+. The decrease in the log K values in the series RNH_3^+, $R_2NH_2^+$, R_3NH^+ is primarily a result of the decreased $\Delta H°$ values, the $T\Delta S$ values remaining essentially constant. Evidence that the binding in these complexes involves hydrogen bonding has been presented and discussed (*9*).

The data in Table 1 show the effect of steric factors in several cases, for example, (i) $(CH_3)_3CNH_3^+$ compared to $CH_3CH_2NH_3^+$ and (ii) $(CH_3CH_2)_2NH_2^+$ compared to the cation **2**. In both cases, the first listed cation has a lower log K value associated with formation of its complex. The smaller log K values result from markedly lower (less exothermic) ΔH values. It is noteworthy that there is little change in the enthalpies of formation for the series of ammonium salts $(CH_3)_nCH_{3-n}NH_3^+$ when n = 0, 1, 2. Evidently, significant steric interactions do not occur until n = 3 [case (i) above].

Table 1. Log K, ΔH, and $T\Delta S$ values for the 1:1 reactions in 100 percent methanol at 25°C of several amine cations with 18-crown-6. Values are the average of four to six experimental determinations. The uncertainties are given as standard deviations.

Cation*	Log K	ΔH (kcal/mole)	$T\Delta S$ (kcal/mole)
NH_4+, I^-	4.27 ± 0.02	−9.27 ± 0.18	−3.5
$CH_3NH_3^+$, I^-	4.25 ± 0.04	−10.71 ± 0.17	−4.9
$CH_3CH_2NH_3^+$, I^-	3.99 ± 0.03	−10.65 ± 0.10	−5.2
$CH_3CH_2CH_2NH_3^+$, I^-	3.97 ± 0.07	−10.06 ± 0.06	−4.6
$(CH_3)_2CHNH_3^+$, I^-	3.56 ± 0.03	−9.65 ± 0.17	−4.8
3, I^-	3.90 ± 0.01	−10.12 ± 0.06	−4.8
$NH_2NH_3^+$, Cl^-	4.21 ± 0.02	−10.43 ± 0.09	−4.7
$HONH_3^+$, Cl^-	3.99 ± 0.03	−9.01 ± 0.21	−3.6
$(CH_3)_3CNH_3^+$, I^-	2.90 ± 0.03	−7.76 ± 0.08	−3.8
$(CH_3)_2NH_2^+$, I^-	1.76 ± 0.02	−6.67 ± 0.23	−4.3
$(CH_3CH_2)_2NH_2^+$, I^-		†	
2, I^-	1.98 ± 0.01	−7.36 ± 0.11	−4.7
$(CH_3)_3NH^+$, I^-		†	
4		†	

*Other results show no significant anion effect upon the thermodynamic quantities: I^-, Br^-, Cl^-, SCN^-, and $CH_3SO_3^-$ have been investigated for several ammonium salts. †The uncorrected heat change in these cases is approximately 100 mcal, $(CH_3CH_2)_2NH_2^+$, or 60 mcal, $(CH_3)_3NH^+$, or **4**, compared to approximately 1300 to 1600 mcal for the $-NH_3^+$ cations and 500 to 600 mcal for $(CH_3)_2NH_2^+$ and **2**. We are unable to calculate reliable log K and ΔH values in these cases; however, extrapolation leads to the conclusions that $-\Delta H$ and log K are small.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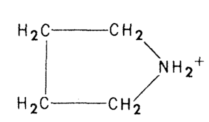
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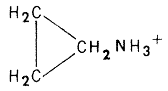
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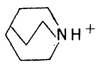
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The higher log K and $-\Delta H$ values for cation **3** compared with $(CH_3)_2CH_2NH_3^+$ are also consistent with reduced steric hindrance in the former case. The formation constants for the cyclic ether complexes are rather sensitive to solvation effects. Going from pure water to pure methanol, the formation constants for the ammonium and methylammonium ions increase by over three orders of magnitude (Table 1) (*9*).

The data presented here provide a basis for relating various structural parameters of protonated organic amines, such as number of hydrogen atoms available for hydrogen bonding, steric effects, type and location of substituent groups, for example, with the log K, ΔH, and $T\Delta S$ values for formation of their host-guest complexes. These relationships have value in (i) understanding the factors that determine the magnitude of log K and how log K changes as ligand and cation parameters are changed, and (ii) establishing stability sequences, which provide a basis for the design and synthesis of ligands for use as models for enzyme action and membrane transport processes.

A sample of compound **1** was obtained from Parish Chemical Company (Provo, Utah). Its purity, determined by calorimetric titration with KBr in absolute methanol, was found to be > 99 percent after purification. The amines were obtained from Aldrich Chemical Company. Amine salts were prepared by adding the anhydrous acid (gas) to an anhydrous ether-amine solution. The precipitated salts were then repeatedly recrystallized from various combinations of anhydrous ethyl ether, absolute ethanol, and absolute methanol, and dried under vacuum. The purity of the amine salts was checked by melting point (in vacuum) and by calorimetric titration with standard crown ether or sodium methoxide solution.

Solutions of 18-crown-6 (0.22*M*) in absolute methanol (< 0.1 percent H_2O) were titrated into methanol solutions of amine salts (0.0075*M*) in a precision titration calorimeter (*9*). Log K, ΔH, and $T\Delta S$ values were calculated from the calorimetric titration data as described previously (*9*). No activity coefficient corrections were made because of the low ionic strengths used (< 0.01*M*) and because there is no satisfactory method available to distinguish between the activity of a protonated amine and a protonated amine-18-crown-6 complex.

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Supersaturated Island Faunas: A Species-Age Relationship for Lizards on Post-Pleistocene Land-Bridge Islands

Abstract. *Lizard faunas on post-Pleistocene land-bridge islands in the region of Baja California were examined for the effect of island age on species diversity. Species diversity and age are significantly correlated; and when the variation in species diversity attributable to area and latitude are removed, the corrected species diversity plotted against island age produces a relaxation or extinction curve. These results provide evidence for supersaturated faunas which are relaxing to lower levels of species diversity consistent with the equilibrium theory of island biogeography.*

The equilibrium theory of island biogeography (*1*) proposes that the number of species in some biotas may be explained by an equilibrium resulting from a balance of immigration by extinction. Although the theory has been broadly applied (*2*) and widely accepted, it has recently been impugned for a lack of conclusive evidence for the existence of an immigration-extinction equilibrium in nature (*3*).

Paradoxically, some of the most convincing evidence for the theory to date comes from apparent nonequilibrium faunas. These are post-Pleistocene isolate faunas, such as on islands that are presumed to represent the dwindling portions of once larger faunas. These isolates were either part of a continental fauna from which they have become separated or were part of an already existing isolate that has been shrinking in size because of the rise of the sea level. The greater-than-expected numbers of species found in some of these faunas, relative to what is predicted for similar faunas in regions of comparable size but with a stable geological past, can be explained by the equilibrium theory. The theory would predict that they are supersaturated and in the process of shrinking or relaxing to equilibrium states with fewer species.

Diamond (*4*) and Terborgh (*5*), working independently in the southwest Pacific and Neotropics, respectively, have reported the existence of supersaturated bird faunas. Further, they have obtained remarkably similar results for relaxation rates dependent on island size, and for equilibrium species numbers. Soulé and Sloan (*6*) earlier provided evidence for supersaturated lizard faunas on islands in the Gulf of California. Subsequently, Case (*7*) produced more evidence for the supersaturated status of some of the same faunas, and also made estimates of the dependence of relaxation rates on island size which corroborate the above results for bird faunas. Some workers nevertheless question whether positive results from such studies are evidence for the equilibrium theory, and have either disregarded them (*3*), or questioned their validity (*8, 9*). For example, Abbott and Grant (*9*) state that there are no undisputed cases of nonequilibrium faunas due to a lack of sufficient time to attain their equilibria.

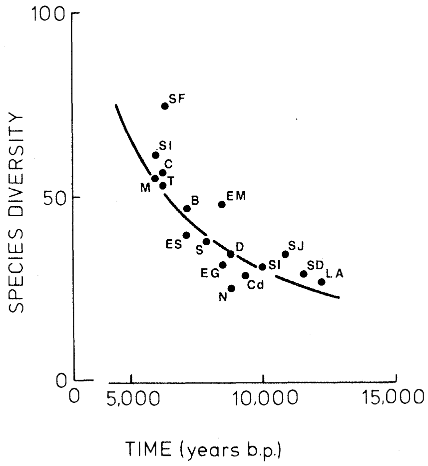

Fig. 1. Species diversity, corrected by partialing out variation due to area and latitude, plotted as a function of isolation time. *SF*, San Francisco; *SI*, San Ildefonso; *SM*, San Marcos; *M*, Mejia; *T*, Tiburon; *B*, Ballena; *EM*, El Muerto; *ES*, Espíritu Santo; *S*, Smith; *D*, Danzante; *EG*, Encantado Grande; *N*, Natividad; *Cd*, Cedros; *C*, Coronado; *SJ*, San Jose; *SD*, San Diego, *LA*, Las Animas.

Such caution is reasonable in that the identification of supersaturated faunas is based on estimates of initial and final (equilibrium) numbers of species as predicted from other faunas of the same taxa and region that are assumed to represent both of the above extremes. Thus evidence for the equilibrium theory from such studies is necessarily indirect. Here I ask whether it is possible to obtain evidence for supersaturated nonequilibrium faunas without making these assumptions.

In previous studies, it was assumed that upper- or post-Pleistocene land-bridge islands became isolated 10,000 years ago (*4, 5*). Actually, isolation times have been variable, occurring between approximately 6,000 and 14,000 years ago as the eustatic sea level rose gradually. Therefore, land-bridge islands became isolated from the mainland at different times and should demonstrate a correlation between time since isolation and the degrees of supersaturation of faunas, given that a sufficient number of the island faunas remain supersaturated. Obtaining a quantitative relationship between island age and species number would provide direct evidence for nonequilibrium supersaturated faunas.

I have tested for such a relationship by considering 17 land-bridge islands in the region of Baja California. These islands were chosen because they are upper- or post-Pleistocene land-bridge islands, their lizard faunas are derived from virtually the same mainland pool (*10*), and the lizard species composition of each is known (*6, 11*). Stepwise multiple regression and correlation were used to determine which of several variables, including time since isolation (age), explain a significant amount of variation in lizard species diversity between islands. Lizard species diversity is here defined as $S = (N/P)100$, where N is the number of species on an island and P is the number