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Genetic Control of an Insect Neuronal Network

Abstract. *Motor activity responsible for the calling song of crickets is generated by a small neuronal network whose output is genetically determined. Genes controlling certain output features are located on the X chromosome. The genetic system involved is polygenic and multichromosomal. In some patterns, genetically derived information is adequate to specify the difference of a single impulse in the output of homologous neurons from different genotypes.*

Although animal behavior is directed to varying degrees by genetic information, the way such information is "read out" remains obscure. The nervous system must figure prominently in this process, and many investigations of neurogenetics are appearing (*1*). Progress has been hindered by difficulties dealing with nongenetic factors affecting behavior, in studying small neurons or large populations of neurons, and in finding a circumscribed behavior amenable to both genetic and neurobiological study. Stereotyped insect behavior patterns, such as cricket singing, which are generated by a small network of large, genetically determined neurons, promise to resolve some of these difficulties.

Field crickets produce several songs for intraspecific communication. The best known is the calling song, broadcast by isolated males to attract receptive females. Although bouts of singing are normally triggered by light-dark cycles, crickets have an endogenous singing rhythm and do not require the environmental cue (*2*). The pattern of motor neuron firing responsible for the calling song is generated by a small group of neurons in two thoracic ganglia. Even when isolated from sensory input, this network can produce a song pattern that is indistinguishable from the normal pattern (*3, 4*). Interneurons and motor neurons involved can be monitored, driven, and filled with dye via intracellular microelectrodes (*4*). Similar neuronal programming circuits control many invertebrate behavior patterns (*5*). Among such circuits, the calling song network is unusual in its degree of independence from sensory input and in the resultant invariability of output.

Since the pattern is generated without reference to the current environment of the animal, the network must be using information previously available. The circuit appears to be sequentially laid down during the latter half of the nymphal life-span and is completed before the final molt to adulthood (*6*). To investigate the contribution of genetic information to this process, cricket species with different songs were hybridized, and the sound pulse and motor unit firing patterns of subsequent generations were analyzed. [Work on sound pulses of the wild type and F_1 was done in collaboration with R. Hoy (*7*).] The results show that (i) the song programming network is under firm genetic control and is buffered from variation in the environment; (ii) the genetic system involved is polygenic and multichromosomal, even for single song features; (iii) genes controlling some features are on the X chromosome, while other features are under autosomal control; and (iv) the precision of genetic control is adequate to specify a difference of a single impulse in the trill patterns of identified homologous motor neurons from different genotypes.

Teleogryllus commodus and *T. oceanicus*, Australian and Polynesian field crickets, produce complex calling songs containing a series of chirps and trills arranged in a repeating phrase (Fig. 1, A and F). Females produce 1500 to 2000 eggs (*2*), and the generation time is about 6 weeks at 35°C. The crickets were hybridized by reciprocal crosses (each species was the maternal parent in one cross and the paternal parent in a second cross). Several hundred first filial generation (F_1) nymphs were raised and crossed with both parental species (F_1 females were sterile). Calling songs (at 24.5° ± 1°C) of wild-type, F_1, and backcross males were recorded on tape and filmed on oscillograph paper (Fig. 1). Several hundred consecutive interpulse intervals (time from onset of one sound pulse to onset of the next) were measured, displayed in histograms and successive interval plots, and statistically characterized (Table 1). Eighteen neuronally determined characteristics of the calling pattern were measured in wild-type and F_1 songs; in the backcrosses, all of these features were scanned visually but only three were treated statistically. Except for two backcross classes (Table 1), conclusions were drawn from ten individuals of each type.

The interval structure of wild-type and hybrid calling song patterns demonstrates that this neuronally generated behavior is determined almost exclusively by genotype. Individuals with different genotypes produced different songs that formed a series of patterns bridging the two wild types (Fig. 1, B–E; Table 1). Despite being raised under different conditions of temperature, diet, light cycle, time of year, and population density, individuals always produced calling patterns corresponding to genotype. The "correct" song for a genotype was produced even if an animal was the first of its type to mature and therefore had heard many "incorrect" songs, but none of its own. Individuals with different genotypes produced different song patterns even if raised under nearly identical environmental conditions. These results agree with earlier observations on acoustical behavior of hybrids (*8*). The ultimate source of information for this programming network appears to be genetic, and this will probably be true for similar neuronal programs.

The number of genes or linkage groups that control the network can be estimated by a classical genetic analysis of the number of classes of backcross individuals. [All F_1 charac-

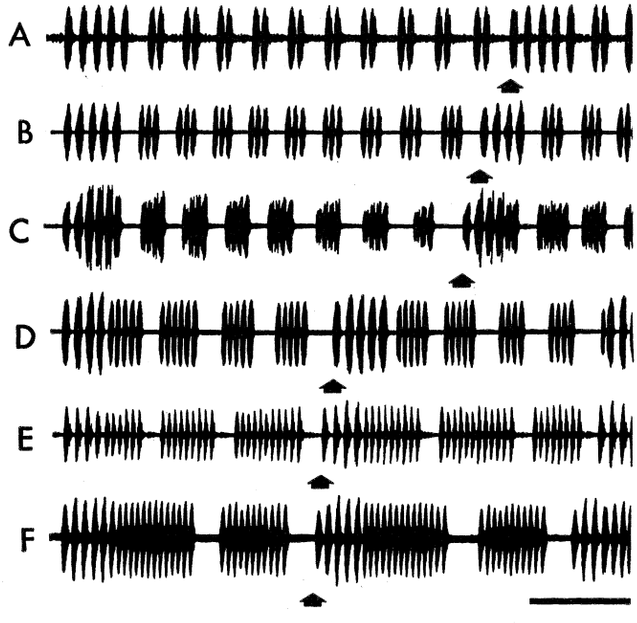
ters were intermediate; there was no simple dominance (*7*).] If an output feature were controlled by a single gene, the backcross should produce two classes of individuals, one like each parental type; increasing numbers of genes would produce an increasingly smooth distribution of the feature in the backcross progeny. In 50 individuals of a single backcross type [O/(O/C), Table 1], three song features—pulses per trill, trills per phase, and intertrill interval—were measured, and the data were plotted in histograms to reveal the number of classes. None of the histograms showed the bimodal distribution consistent with a single gene hypothesis, and therefore these characteristics appear to be under polygenic control. When all of the song pattern features are considered, it is clear that the genetic information for even this limited neuronal network is widely distributed through the genome.

It is possible to localize genes controlling particular song features to a single chromosome when these genes are located on the sex chromosomes. Male crickets have no Y chromosome, so all genes located on the X chromosome (received from the maternal parent) are unduplicated (*8, 9*). If two species are crossed reciprocally, the two types of F_1 males will be genetically identical, within intraspecific variation, except for their X chromosomes. Therefore, phenotypic differences in the songs of these males can be attributed to genes located on the X chromosome [*T. oceanicus* has 29 chromosomes (*9*)].

Several classical criteria are available for distinguishing sex-linked characters from those that are not sex-linked. (i) Features influenced by sex-linked genes should be different in the two types of F_1 song patterns. (ii) Each pattern should be more similar to that of males of the maternal species. (iii) If both types of F_1 males are crossed to females of one parental species, the difference in songs should disappear in the two types of male backcross offspring. (iv) Songs of backcross males should still be more similar to those of males of the maternal species. (v) Features that are not sex-linked should not be significantly different in the two types of F_1 males and should be intermediate between the parental types both in F_1 and in backcross progeny.

Eighteen song pattern characteristics were examined for sex linkage in F_1 males, and several appeared to be sex-linked (*7*); three features—intertrill interval, pulses per trill, and trills per phrase—were analyzed in the four types of backcross offspring (Table 1). Intertrill interval is influenced by sex-linked genes whereas the other two features are not. (A more remote but unexcluded possibility is inheritance via the egg cytoplasm.) Characteristics of *Drosophila* courtship song have also been reported to be sex-linked (*10*). Since some features of cricket song are sex-linked and others are not, the genetic system is multichromosomal as well as polygenic. Evidently, this is true even of single features of songs, since the sex-linked characters are also influenced by autosomal genes.

An unusual advantage of studying



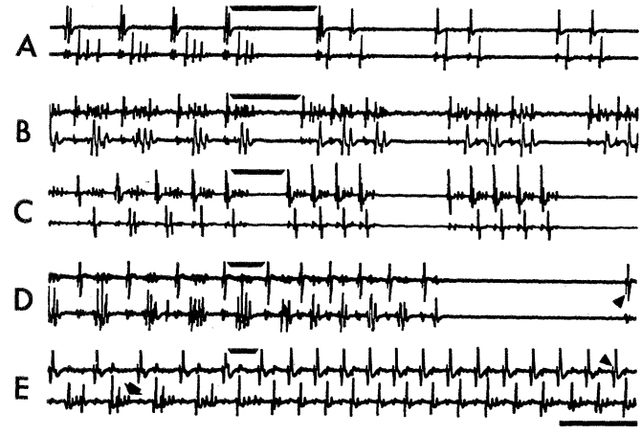


Fig. 1 (left). Sound pulse patterns in the calling song of *Teleogryllus* wild types and hybrids. Each record begins with a single 4 to 6 pulse *chirp*, followed by a series of *trills* containing from 2 to 14 sound pulses, depending on genotype. Chirps and trills are arranged in a repeating *phrase*. Records start with a complete phrase, and arrows mark the onset of the second phrase. Records are as follows: A, wild type, *T. oceanicus*; B, backcross, *T. oceanicus* ♀ × F_1 ♂ (shown in C); C, F_1, *T. oceanicus* ♀ × *T. commodus* ♂; D, F_1, *T. commodus* ♀ × *T. oceanicus* ♂; E, backcross, *T. commodus* ♀ × F_1 ♂ (shown in D); F, wild type, *T. commodus*. Song patterns are strictly determined by genotype. Most hybrid features are intermediate between corresponding parental features; for example, number of sound pulses per trill and number of trills per phrase. The bar at bottom shows 0.5 second.

Fig. 2 (right). Motor unit firing patterns responsible for the calling song of *Teleogryllus* wild types and hybrids. Records are as follows: A, wild type, *T. oceanicus*; B, backcross, *T. oceanicus* ♀ × F_1 ♂ (shown in C); C, F_1, *T. oceanicus* ♀ × *T. commodus* ♂; D, backcross, *T. commodus* ♀ × F_1 ♂ (shown in C); E, wild type, *T. commodus*. Traces show muscle action potentials recorded from single, identified fast units, and elicited by single motor neuron impulses (triangles, D and E). The upper trace shows a wing opener unit (unit 1, subalar muscle), and the lower trace shows a wing closer unit (unit 2, promotor muscle); wing closing produces the sound pulse. The portion of the phrase shown is the transition (bars) from the chirp (left of bars) to the sequence of trills (right of bars). Motor unit activity corresponds to, and determines, the sound pulse patterns (Fig. 1). Details of differences in firing patterns of homologous single motor neurons from different genotypes are seen. (i) The transition interval (bar) decreases steadily from *T. oceanicus* (A) to *T. commodus* (E). (ii) Motor unit bursts per trill increase from 2 (A) to 14 (E). Genetic information is precise enough to specify a difference of one burst between the trills of *T. oceanicus* (A) and the backcross (B). For many units, this difference is only a single impulse. In some traces, the smaller promotor unit (arrow) partially obscures the recording. The bar at the bottom shows 100 msec.

Table 1. *Teleogryllus* hybrid song pattern features. Abbreviations are as follows: N, number of individuals; $\bar{X}$, mean; S.D., standard deviation; n, number of observations; O, *T. oceanicus*; C, *T. commodus*. The maternal parent is listed first in each cross. Statistical procedures have been described (*7*).

Genotype		N	Intertrill interval			Pulses per trill			Trills per phrase		
			$\bar{X}$ (msec)	S.D. (msec)	n	$\bar{X}$ (msec)	S.D. (msec)	n	$\bar{X}$ (msec)	S.D. (msec)	n
Wild type	O	10	122.8	14.1	813	2.0	0.1	250	9.4	2.3	100
Backcross	O/(O/C)	50	123.1	17.8	4276	2.9	0.5	3988	6.6	2.4	630
	O/(C/O)	10	121.2	21.2	453	3.0	0.9	541	7.8	1.2	66
F_1	O/C	10	136.8	23.6	837	4.5	1.5	194	4.2	1.0	100
	C/O	10	154.0	38.8	693	4.9	2.0	200	4.8	1.2	100
Backcross	C/(O/C)*	2	174.8	43.2	343	6.9	2.8	424	3.6	1.2	112
	C/(C/O)	10	158.4	36.8	389	7.0	3.1	459	3.2	1.2	133
Wild type	C	10	160.9	60.9	119	10.7	5.3	147	2.3	1.2	100

* Data are relatively unreliable due to small number of individuals.

acoustical behavior is that sound pulse patterns, easily recorded from large numbers of animals, are precise monitors of a specific neuronal network. To establish the accuracy of this monitor and to examine fine structure of output patterns, wires were implanted in identified muscles, and action potentials were recorded during calling songs of unrestrained animals (Fig. 2) (*11*). Most of the muscles involved have only one to three fast, hierarchically arranged motor units, each of which is activated in a one-to-one manner by a single motor neuron. Therefore, the muscle action potentials reflect the discharge of single motor neurons, and with fortunate electrode placement the neuronal potentials can also be seen (Fig. 2, D and E). Action potentials of specific wing opener and wing closer muscle units were recorded from wild-type, F_1, and backcross males. For each wing stroke, potentials occur either singly or in short bursts of one to three impulses. The timing of the bursts determines the pattern of sound pulses, and the number of potentials per burst determines the intensity of the sound pulse. For example, the loud chirp pulses usually correspond to multiple impulse bursts, while the softer trill pulses are often produced by only a single potential in a given motor neuron (Fig. 2).

Motor output undergoes the same change from genotype to genotype as does sound pattern, but genetically determined differences in the discharge of single neurons can be resolved. Two examples illustrate this. (i) In *T. oceanicus* there is a substantial pause between the last pulse of the chirp and the first pulse of the subsequent trill, whereas in *T. commodus* the chirp and the first pulse of the trill are continuous. This difference is also seen in the firing pattern of single neurons in the wild types and hybrids, and indicates either that the homologous neurons constructed by different genotypes have different properties, such as rate of adaptation, or that new circuit elements such as inhibitory neurons are present. (ii) Motor units reflect the shift in number of sound pulses per trill from two in *T. oceanicus*, to three in the nearest backcross, to even more in genotypes approaching *T. commodus*. For most single motor units, activity corresponding to a trill pulse is only a single impulse, so the difference between the output of homologous neurons in *T. oceanicus* and in the backcross is only a single action potential per trill. Therefore, genetically stored information is capable of specifying the output of single neurons with resolution approaching the theoretical maximum—single impulse increments.

How can a genetically determined neuronal network reveal the relation between genes and neurons? Two different questions are involved. (i) What aspects of the network, such as intercellular connections or the set points of intracellular variables, are genetically specified to control the output pattern? This question can be answered by comparing structure and physiology of homologous neurons in networks constructed according to different genetic specifications. Analysis could be extended to the biochemical level (*12*), since the cell bodies form the ganglion cortex and can be identified and removed without difficulty. (ii) What do single genes contribute to construction of the network? Effects of single genes could be isolated by induction of mutations or by meticulous selection. Because of the fecundity and short generation time of these crickets and the ease of scanning acoustically for network malfunctions, these classical genetic methods appear feasible.

Construction of the neuronal programming network responsible for cricket calling song is determined by genetic information. The genetic system involved is polygenic and multichromosomal, even for single output features. Genes controlling some features of the pattern are located on the X chromosome. The genetic information is precise enough to specify a difference of a single impulse between trill patterns of homologous motor neurons from different genotypes.

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