

# Vocalizations in Newborn Mice: Genetic Analysis<sup>1</sup>

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Two kinds of vocalizations are produced by newborn mice: whistles (between 50 and 150 ms in length), having a narrow bandwidth in each strain that ranges from 30 to 90 kHz; and clicks, which are shorter (about 1 ms) and have a larger bandwidth. These vocalizations were individually recorded in 1-day-old pups from seven inbred strains of laboratory mice, at two temperatures ( $23 \pm 0.5$  and  $15 \pm 0.5^\circ\text{C}$ ). The numbers of clicks and whistles were counted under these two conditions. Moreover, the length and frequencies at the beginning, apex, and end of the whistles were measured during the  $15^\circ\text{C}$  condition. Correlations, including several components—additivity, epistasis (between homozygous loci), and maternal environment—were calculated between the characteristics of the whistles during the  $15^\circ\text{C}$  condition. Clicks and whistles were also counted from 1 to 8 days of age during the  $15^\circ\text{C}$  condition. The numbers of clicks and whistles were age dependent, with a decrease from day 1 to day 8 for the clicks and a consistent production of whistles. A quantitative genetic analysis was also performed on the 1-day-old pups from the Mendelian generations produced by the inbred strains most contrasting for the number of whistles produced in the cold condition: NZB/BINJ and CBA/H. The heterozygous genotype of the mother induced an increment of the number of whistles. Moreover, a significant part of the additive variance was suspected from the first design, and found with the second one, for this variable. Quantitative genetic analysis showed significant dominance and epistasis between homozygous loci and homozygous and heterozygous loci. This points to multigenic correlates for the number of whistles in this population. The significant additive values for all the variables recorded during the  $15 \pm 0.5^\circ\text{C}$  condition and for the number of whistles produced during the  $23 \pm 0.5^\circ\text{C}$  condition are compatible with an effect that indicates neither directional nor stabilizing selection. This result is examined in the light of the multichannel sensorial process implicated in maternal behavior in mice.

**KEY WORDS:** Vocalizations; ultrasounds; development; newborn; mice; NZB/BINJ.

## INTRODUCTION

Zippelius and Schleidt (1956) provided the first evidence for ultrasound production among the vocal-

izations produced by newborn rodents. Bell *et al.* (1972) have pointed out the physical complexity of these vocalizations, which include whistles and clicks. Whistles are purely ultrasonic: they range within a large bandwidth, from 30 to 90 kHz, depending on the individual. They are emitted under

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1  $\mu$ bar, and their duration is between approximately 50 and 150 ms. Clicks are shorter, less than 1 ms, and have a large bandwidth (Bell *et al.*, 1972), including both ultrasonic and audible components. Whistles and clicks both occur at the expiration phase (Roberts, 1975). They are emitted in outbursts and are closely associated (Elwood and McCawley, 1983). These vocalizations occur during stressful situations in rats and mice, such as hunger (Jans and Leon, 1983), cold (Okon, 1970; Bell *et al.*, 1972; Noirot, 1972; Robinson and d'Udine, 1982), handling (Bell *et al.*, 1971), and unfamiliar odors (Oswalt and Meier, 1975).

Ultrasound production is considered to have a communication function essential for the pups' survival (Noirot, 1972; Smith, 1976; Elwood and McCawley, 1983). These authors and others have stressed the implications of ultrasound production in retrieving or maternal behavior, as follows. Pups are unable to thermoregulate from birth to 6 days of age, and consequently, maternal retrieving of the pups to the nest and subsequent lactating position is necessary to ensure a constantly high body temperature. Visual and acoustic stimulation provides the distal cues that initiate retrieving and maternal behavior. However, Smotherman *et al.* (1974) demonstrated that the presence of auditory cues elicited shorter retrieving latencies only when the female was aroused by olfactory cues. Experiments with inbred strains of mice also challenged the conclusions about auditory cues maintaining maternal behavior. A positive interstrain correlation was obtained between the number of ultrasounds produced by the pups (Roubertoux *et al.*, 1984) and the different measures of retrieving behavior (Carrier *et al.*, 1982) of their mothers. This association disappeared, however, when the ability of the mother to perceive ultrasounds was considered (Cohen-Salmon *et al.*, 1985). Using a cross-fostering design, pups from a high-emitting strain (C57BL/6J) were not retrieved more quickly than pups from a low-emitting strain (XLII) by well-hearing mothers (as shown by electrophysiological methods) from the C57BL/6J strain.

The question of the function of ultrasound production in maternal behavior thus remains unanswered. A genetic analysis, however, should contribute to an answer, providing information on its adaptive value. Are the number of ultrasounds or frequency differences correlated with one or several genes and do differences follow a heterotic

mode of inheritance? Lerner (1954) and Bruell (1964) analyzed heterosis as a consequence of polygenic inheritance and also as a characteristic of those traits which contribute to the fitness of the organism having been subjected to pressures of selection. The complementary dominance hypothesis states that genes with dominant effects are beneficial, whereas genes with recessive effects are deleterious when they are carried in double doses. Are the strain differences between poor emitters and high emitters due to additivity, dominance, or epistasis? Very little is known about ultrasound production in newborns. Several investigators have identified mutations that lowered the number of ultrasonic productions (D'Udine *et al.*, 1982; Bolivar and Brown, 1994). To the best of our knowledge, only one genetic analysis has been carried out using newborn pups derived from Mendelian crosses (Hahn *et al.*, 1987). A directional dominance was observed for the high number of whistles, suggesting that this trait was "strongly related to fitness and has been under strong directional selection" (Hahn *et al.*, 1987, p. 164). On the other hand, the one locus hypothesis was not rejected for this variable and it would have been surprising for a trait involved in the preservation of the species to be subjected to the hazards of a possible mutation. For the other measures (frequencies and duration of the whistles), the findings of Hahn *et al.* were not clear-cut. Are those findings restricted to the strains used in this experiment since the observed difference for the number of whistles between the strains was so small?

We present here the screening of seven different inbred strains for the number of clicks and whistles, as well as the duration and frequencies of the whistles. We have also analyzed the correlations between these measures (experiment 1) and the development of the number of clicks and whistles during the first 8 days after the birth (experiment 2). The two strains showing the greatest differences for the number of whistles were subsequently selected for a genetic analysis with the newborns derived from the Mendelian crosses (experiment 3).

## MATERIALS AND METHODS

*Rearing Conditions.* Mice were maintained under the following general conditions: temperature,  $23 \pm 0.5^\circ\text{C}$ ; photoperiod, 12:12 with lights

on at 0800; food, im UAR; and weaning, at  $29 \pm 2$  days. The females were placed in groups of three per cage, when they were 60 days old, with a male. About 3 days before parturition, each female was isolated in a transparent cage ( $48 \times 18 \times 17$  cm) containing 1 L sawdust.

*Vocalization Recordings and Measurements.*

For all experiments, the cages were visually inspected at 0800 and 1930. The vocalizations produced by the pups were recorded the day after the litter was first seen. Each pup was then placed on a glass dish. The recording started 10 s after the pup had been put on the dish, to avoid the calls induced by handling, and lasted 60 s. Vocalizations were recorded on an Ampex 707 tape recorder, at a tape speed of 154 cm/s, enabling the recording of frequencies from 4 Hz to 100 kHz. The filter was a Belin-type F260 with a high-pass filter set at 20 kHz. A Bruel and Kjaer microphone-cathode follower 2614 with capsule 4130 and bandwidth from 20 kHz to 100 kHz was placed at  $0^\circ$  incidence and 3.5 cm above the dish, i.e., about 2.5 cm above the head of the pup.

Experiment I included two steps. The first one was a screening of strain and sex differences. The pup was placed on a glass dish containing sawdust from its own cage and kept at either  $23 \pm 0.5^\circ\text{C}$  (hereafter labeled the  $23^\circ\text{C}$  condition) or  $15 \pm 0.5^\circ\text{C}$  ( $15^\circ\text{C}$  condition). The recordings were performed as indicated above. The aim of the second step was to calculate genetic correlations between the measures performed for the vocalizations. It was thus necessary to obtain all the measures for the same pup. The pup was placed in the  $23^\circ\text{C}$  condition, then marked with India ink for identification and placed again in the cage with its mother. Two hours later, the same pup was placed in the  $15^\circ\text{C}$  condition. The recordings were performed as indicated above.

An aural count was preferred to an automatic one. After reducing the tape speed eight times, one of us (P.L.R.) counted the vocalizations. They were counted, blind, twice for each recording. For each individual, the score was the average of the two measures. Two kinds of vocalizations were counted, whistles and clicks, the latter recognized as short vocalizations (Zippelius and Schleidt, 1956; Noirot, 1972).

The characteristics of the individual whistles were also measured during the  $15^\circ\text{C}$  condition. The signals were transcribed onto charts via a Kay Ele-

metric 7029A Sona-Graph sound spectrum analyzer, with reduced tape speed to 21.8 cm for 1 s. The beginning, apex, and ending frequencies, as well as the duration, of the whistles were measured, blind. The first two whistles produced were measured for each pup. In NZB/BINJ and XLII, where several individuals, respectively, did not emit whistles within the 60 s, it was necessary to lengthen the recording session to collect two whistles for the frequency analyses. These pups were counted as zero for the number of whistles.

The numbers of whistles and clicks in the second experiment and the number of whistles in the third were counted as indicated for the first experiment.

#### EXPERIMENT 1: ULTRASOUND PRODUCTIONS AND CORRELATIONS IN SEVEN STRAINS

*Animals.* The mice used in this experiment belonged to seven inbred strains. They were derived from identified breeders from the CSEAL, CNRS (Orléans La Source), for A/J, CBA/H, DBA/2J, and XLII and from Jackson Laboratory (Bar Harbor, Maine) for C57BL/6J, BALB/cJ, and NZB/BINJ. All strains had been maintained in URA CNRS 1294, under a brother  $\times$  sister mating breeding protocol for several generations. For the screening step, the numbers of clicks and whistles were counted in 15 pups per strain, during the  $23^\circ\text{C}$  condition. These vocalizations were also counted during the  $15^\circ\text{C}$  condition in other pups (identical strains and sample size). Length and frequencies of the whistles have been obtained from another sample (10 pups per strain). All these samples are labeled Population I. Each sample included about 50% males in each condition. For the second step, we measured the number of clicks and whistles during the 23 and  $15^\circ\text{C}$  conditions, and length and frequencies of whistles during the  $15^\circ\text{C}$  condition, in five pups per strain. Each pup belonged to a different litter in each strain (Population II).

*Statistical Analysis and Genetic Design.* The numbers of whistles and clicks were considered separately for the two conditions, the 15 vs.  $23^\circ\text{C}$  condition, using a two-way ANOVA (condition and strains as main factors). Replication of the results, by comparison of the subsamples of Population I with Population II, and a possible effect of the gender, were also tested in Population I.

Estimations of the genetic contribution and correlations were obtained according to the method of Hegmann and Possidente (1981), in which the component of the variance among strains divided by the total component of variance between strains plus within strains (B + W) is twice the proportion of an estimation of additive variance. This value might be, however, an overestimate of the additive component, especially when the components due to epistasis between loci with homozygous allelic forms and maternal components have significant effects. This overestimate also inflates the magnitude of the genetic correlation. Due to the small number of subjects per strain, more conservative estimates of the standard errors were derived from Hegmann and Possidente (1981).

They are, respectively,

$$SE(\alpha_n) = \frac{k(n-1)}{k(k-1)-2} \sqrt{\frac{2}{k-1} \frac{k(n-1)+k-3}{k(n-1)-4} \left[ \alpha(1-\alpha) + \frac{(1-\alpha)^2}{2n} \right]}$$

$$SE(r_\alpha) = \frac{1-r_\alpha^2}{\sqrt{2}} \sqrt{\frac{SE(\alpha_n(x))}{\alpha_n(x)} \frac{SE(\alpha_n(y))}{\alpha_n(y)}}$$

where  $\alpha$  is the ratio  $V_{[d]+[i]+[me]}/V_{[p]}$ . The estimated components are in brackets:  $d$  is the additive component,  $i$  the interaction between homozygous loci,  $me$  maternal effects, and  $p$  the phenotypic variance. The correlation due to  $[d]$ ,  $[i]$ , and  $[me]$ , between two variables  $x$  and  $y$ , is  $[r_\alpha]$ . The numbers of subjects per strain and the numbers of strains are  $(n)$  and  $(k)$ , respectively. The  $\alpha$  values were calculated in subsamples of Population I, for the number of vocalizations, in the two conditions and for the measures of the whistles in the cold condition. The  $\alpha$  and  $r_\alpha$  values were calculated in Population II for all the measures and in a subsample of Population I for the four measures of the whistles. Conservative estimates of the reliabilities of the measures were obtained from the highest phenotypic correlation between the strain mean values in Population II that was used for the estimation of genetic correlations.

**Results and Discussion.** The mean values for measures, strains and populations are shown in Table I. The replication effect never reached the  $p < 0.10$  level of significance, and this point is in agreement with quite similar  $F$  and  $\alpha$  values. Gender was involved neither alone nor in interaction ( $F < 1.00$ ) in the measures performed the day after birth. Consequently, males and females were pooled for the following analyses. The recording conditions (23

vs. 15°C) had no effect on the numbers of clicks and whistles, either alone or in interaction with strain. The strain effect alone was significant for the number of whistles in the two conditions and for clicks in the cold condition only ( $F$  values are shown in Table I). The beginning, apex, and ending frequencies, and the duration of the whistles that were recorded in the cold condition and measured on the charts, were strain dependent and well replicated.

The reliability of the measures, under the conditions used with Population II was high, except for whistle length, which exhibited a lower value, as shown in Table II. The  $\alpha$  values were significant for the duration of the whistles and their frequencies, and for the number of whistles recorded in the 15°C condition, in Populations I and II.

The  $r_\alpha$  is the common part of  $V_\alpha$  in the two variables. The estimation of  $r_\alpha$  makes sense only when  $V_\alpha$  differs from zero in the two variables of each pair. Significant  $r_\alpha$ , sometimes higher than zero, can be obtained with one nonsignificant  $V_\alpha$  for one of the variables of the pair, and they must be considered as artifacts. Consequently, only the pairs in which the two variables present this condition are shown in Table II. These values are replicable, as shown from the measures that are available from Population I.

## EXPERIMENT 2: AGE FUNCTION FOR THE NUMBER OF WHISTLES AND CLICKS IN SEVEN STRAINS

**Animals.** The number of whistles and clicks was recorded during the 15°C condition, from the day after the litter was first seen to 8 days of age, and counted as indicated above. The pups belonged to the seven inbred strains used in experiment 1. Six measurements were made *per day* and *per strain*: six litters were employed *per strain* and one pup *per strain* was taken at random per litter during 8 days for recordings. The gender was not considered here either, since no significant sex effect had been found in experiment 1. The results were analyzed according to a two-way ANOVA (strain and days as main factors), after a log transformation to homogenize the variances.

**Results and Discussion.** The median numbers of clicks and whistles during the first 8 days after birth are shown in Figs. 1 and 2. The strain, the day, and their interactions were significant for the

Table 1. Strain Comparisons and  $\alpha$  Ratio ( $\pm$ SE) for Vocalizations in 1-Day-Old Mice<sup>a</sup>

	Strain								F	$\alpha \pm$ SE
	A/J	C57BL/6J	CBA/H	DBA/2J	BALB/cJ	NZB	XLII			
<b>Population I</b>										
15°C condition										
No. clicks; n=15	22.50 $\pm$ 3.71	21.10 $\pm$ 4.02	41.02 $\pm$ 5.10	25.40 $\pm$ 7.02	29.81 $\pm$ 8.98	0.60 $\pm$ .03	0.80 $\pm$ .12	7.65**	NS	
No. whistles; n=15	59.80 $\pm$ 4.98	26.40 $\pm$ 4.22	59.20 $\pm$ 8.46	46.60 $\pm$ 6.07	52.80 $\pm$ 6.10	2.95 $\pm$ 1.20	2.00 $\pm$ 0.51	24.06**	0.61 $\pm$ 0.20***	
Whistle length (ms); n=10	65.80 $\pm$ 4.01	72.40 $\pm$ 4.12	43.40 $\pm$ 3.14	51.70 $\pm$ 2.10	95.10 $\pm$ 2.69	25.20 $\pm$ 0.06	77.50 $\pm$ 6.76	21.61**	0.63 $\pm$ 0.15***	
Whistle beginning (kHz); n=10	76.80 $\pm$ 0.94	78.80 $\pm$ 0.44	56.30 $\pm$ 0.79	71.20 $\pm$ 0.74	68.90 $\pm$ 0.46	56.00 $\pm$ 3.09	56.50 $\pm$ 2.24	32.57**	0.73 $\pm$ 0.13***	
Whistle apex (kHz); n=10	81.30 $\pm$ 0.89	82.00 $\pm$ 0.47	65.50 $\pm$ 0.78	71.20 $\pm$ 0.94	71.60 $\pm$ 1.38	56.40 $\pm$ 3.04	56.70 $\pm$ 2.31	41.31**	0.78 $\pm$ 0.11***	
Whistle end (kHz); n=10	62.00 $\pm$ 0.96	60.10 $\pm$ 1.85	61.70 $\pm$ 0.84	59.90 $\pm$ 1.05	54.90 $\pm$ 0.78	55.60 $\pm$ 3.07	42.90 $\pm$ 1.16	17.98**	0.58 $\pm$ 0.16**	
23°C condition										
No. clicks; n=15	20.20 $\pm$ 5.75	28.22 $\pm$ 11.30	16.00 $\pm$ 4.12	18.40 $\pm$ 2.30	35.01 $\pm$ 13.13	9.45 $\pm$ 8.39	6.22 $\pm$ 3.71	1.24	NS	
No. whistles; n=15	44.60 $\pm$ 5.71	66.80 $\pm$ 26.34	56.80 $\pm$ 20.84	41.00 $\pm$ 7.56	61.60 $\pm$ 25.53	2.80 $\pm$ 1.66	9.20 $\pm$ 4.28	2.18	NS	
<b>Population II</b>										
15°C condition										
No. clicks; n=5	23.60 $\pm$ 4.20	18.00 $\pm$ 5.93	39.00 $\pm$ 5.71	29.80 $\pm$ 6.92	26.40 $\pm$ 10.13	0	0.40 $\pm$ 0.24	6.31**	NS	
No. whistles; n=5	57.60 $\pm$ 4.22	25.40 $\pm$ 4.13	69.20 $\pm$ 6.45	45.40 $\pm$ 6.08	51.40 $\pm$ 8.32	2.20 $\pm$ 1.32	1.40 $\pm$ 0.87	27.28**	0.69 $\pm$ 0.16***	
Whistle length (ms); n=5	71.80 $\pm$ 1.14	66.60 $\pm$ 6.38	46.80 $\pm$ 2.60	56.00 $\pm$ 2.21	161.60 $\pm$ 8.62	28.40 $\pm$ 7.05	86.20 $\pm$ 5.40	51.74**	0.81 $\pm$ 0.11***	
Whistle beginning (kHz); n=5	79.00 $\pm$ 0.94	80.20 $\pm$ 2.67	66.60 $\pm$ 1.38	71.40 $\pm$ 1.29	68.40 $\pm$ 0.74	51.20 $\pm$ 2.26	58.80 $\pm$ 2.17	41.46**	0.77 $\pm$ 0.13***	
Whistle apex (kHz); n=5	83.40 $\pm$ 0.25	81.40 $\pm$ 0.06	66.80 $\pm$ 1.40	70.80 $\pm$ 1.83	72.80 $\pm$ 2.33	50.30 $\pm$ 1.50	59.20 $\pm$ 2.03	54.52**	0.82 $\pm$ 0.11***	
Whistle end (kHz); n=5	61.00 $\pm$ 1.81	62.30 $\pm$ 3.21	64.80 $\pm$ 2.33	58.00 $\pm$ 1.05	55.00 $\pm$ 1.42	49.40 $\pm$ 0.41	45.20 $\pm$ 1.38	13.71**	0.51 $\pm$ 0.19*	
23°C condition										
No. clicks; n=5	18.80 $\pm$ 5.48	23.60 $\pm$ 8.68	15.60 $\pm$ 4.13	15.60 $\pm$ 1.93	28.80 $\pm$ 10.13	5.80 $\pm$ 4.82	5.20 $\pm$ 3.17	1.98	NS	
No. whistles; n=5	41.20 $\pm$ 4.66	56.20 $\pm$ 21.78	52.40 $\pm$ 18.59	35.80 $\pm$ 7.09	39.40 $\pm$ 12.60	4.60 $\pm$ 1.56	7.60 $\pm$ 2.94	2.71*	NS	

<sup>a</sup> n: number of pups per strain.  
 \* p < 0.05.  
 \*\* p < 0.01.  
 \*\*\* p < 0.001

**Table II.**  $r_a$  ( $\pm$ SE) Among Number of Clicks and Whistles, Duration, and Frequency of Whistles (15°C Condition) and Number of Clicks and Whistles (23°C Condition), in Newborn Mice;  $n = 5$  per Strain (Roman Numerals);  $n = 10$  per Strain (Italics)<sup>a</sup>

	15°C condition						23°C condition	
	No. clicks	No. whistles	Whistle length	Whistle beginning	Whistle apex	Whistle end	No. clicks	No. whistles
15°C condition								
Number of clicks	<b>0.82</b>	NS	NS	$0.65 \pm 0.13^{***}$	$0.64 \pm 0.12^{***}$	$0.94 \pm 0.04^{***}$	NS	NS
Number of whistles		<b>0.82</b>	NS	$0.60 \pm 0.09^{***}$	$0.63 \pm 0.07^{***}$	$0.86 \pm 0.06^{***}$	NS	NS
Whistle length			<b>0.62</b>	$0.23 \pm 0.11^*$	$0.33 \pm 0.08^{***}$	$0.14 \pm 0.06^*$	NS	NS
				$0.41 \pm 0.12^{**}$	$0.47 \pm 0.18^{**}$	$0.36 \pm 0.06^{***}$		
Whistle beginning				<b>0.92</b>	$0.99 \pm 0.00^{***}$	$0.78 \pm 0.07^{***}$	NS	NS
					$0.99 \pm 0.00^{***}$	$0.70 \pm 0.08^{***}$		
Whistle apex					<b>0.93</b>	$0.74 \pm 0.07^{***}$	NS	NS
						$0.65 \pm 0.08^{***}$		
Whistle end						<b>0.89</b>	NS	NS
23°C condition								
Number of clicks							<b>0.81</b>	NS
Number of whistles								<b>0.89</b>

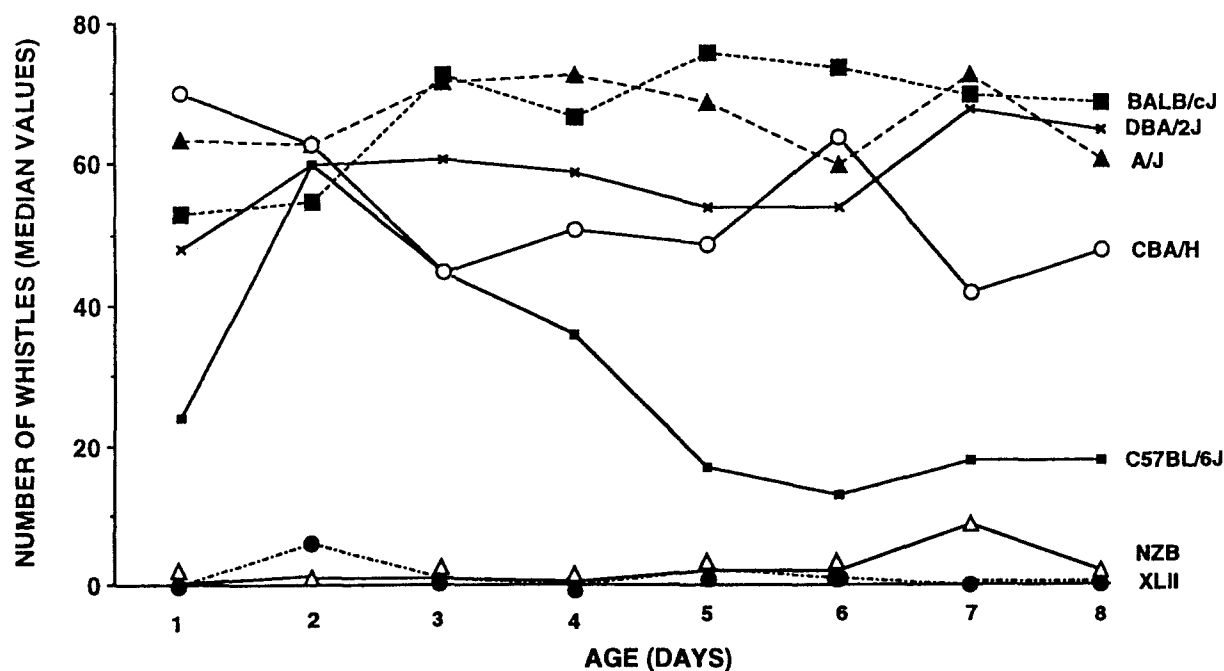
<sup>a</sup> Reliability coefficients are shown on the diagonal (boldface).

\*  $p < 0.05$ .

\*\*  $p < 0.01$ .

\*\*\*  $p < 0.001$ .

<sup>b</sup> NS, nonsignificant or noncalculated values when one of the  $\alpha$  ratio of the pair was not significant.



**Fig. 1.** Median number of whistles produced by newborn mice in seven inbred strains (recording, 60 s; 15°C condition), from the day after the litter was first seen (indicated day 1) to day 8 of age.

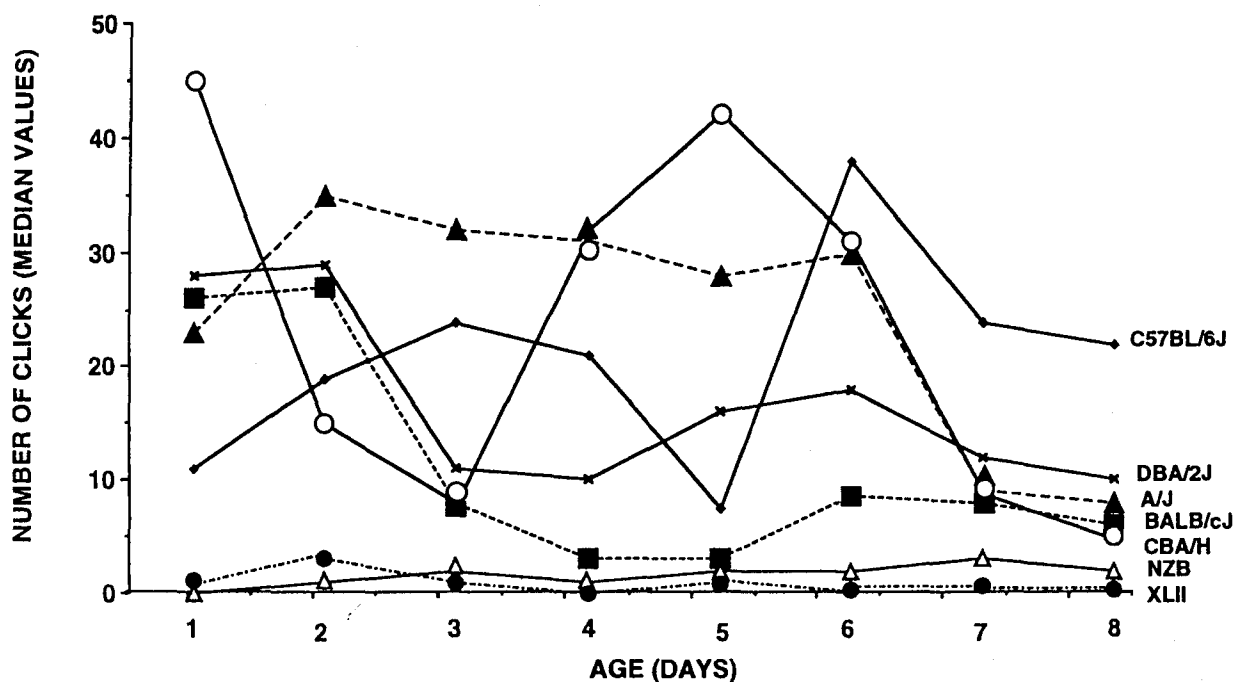


Fig. 2. Median number of clicks produced by newborn mice in seven inbred strains (recording, 60 s; 15°C condition), from the day after the litter was first seen (indicated day 1) to day 8 of age.

number of clicks ( $F = 30.32$ ,  $df = 6,41$ ,  $p < 0.001$ ;  $F = 3.421$ ,  $df = 7,41$ ,  $p < 0.01$ ;  $F = 2.216$ ,  $df = 28,41$ ,  $p < 0.025$ , respectively), whereas the two main factors reached significance only for the number of whistles ( $F = 2.351$ ,  $df = 6,41$ ,  $p < 0.05$ ;  $F = 7.719$ ,  $df = 7,41$ ,  $p < 0.01$ , respectively). Both clicks and whistles were age dependent. The number of clicks decreased for all strains by day 8, except for C57BL/6J. A quite different picture was observed for the number of whistles, since it remained constant during the 8 days, for the low and high emitters, except for C57BL/6J.

### EXPERIMENT 3: SEGREGATION ANALYSIS WITH NUMBER OF WHISTLES

The marked contrast found here between CBA/H (H) and NZB/BINJ (N) for the number of whistles replicated our previously published results (Roubertoux *et al.*, 1984; Cohen-Salmon *et al.*, 1985). Moreover, this contrast was stable over the first week of life. A quantitative genetic analysis, dealing with the number of whistles during the cold condition, was therefore performed employing these strains.

*Genetic Design.* Mendelian crosses were derived from the H and N parental strains, the reciprocal  $F_1$ 's,  $NH_{F_1}$  and  $HNF_1$ ; two backcrosses with H,  $NH \times H$  and  $H \times NH$ ; two backcrosses with N,  $NH \times N$  and  $N \times NH$ ; and two intercrosses,  $HN \times NH$  and  $NH \times HN$ . The latter two did not differ and were pooled. Examination of the variances in the nonsegregating generations showed a crude heterogeneity, due mainly to the small variance in the N strain. A raw data transformation was thus required and we used the HOMAL program (Crusio, 1990). The cubic-root transformation homogenized the variances in the parental strains and their reciprocal  $F_1$ 's, as was shown by the lowest value provided by the Bartlett test [ $\chi^2_{(3)} = 1.027$ ]. The components of the mean differences were then analyzed using the Mather and Jinks (1971) method. The best-fitting model was selected using the Kerbusch *et al.* (1981) procedure. Eight parameters were tested: [ $m$ ] mean, [ $d$ ] additivity, [ $h$ ] dominance, [ $i$ ] interaction between homozygous loci, [ $j$ ] interaction between homozygous and heterozygous loci, [ $l$ ] (interaction between heterozygous loci, [ $dm$ ] contribution of the homozygous mother, and [ $hm$ ] contribution of the heterozygous

**Table III.** Number of Whistles Measured in the Cold Condition: Sample Sizes, Mean Values (Untransformed), and Variances for the NZB/BINJ and CBA/H Parental Strains and Their Derived Mendelian Crosses and Coefficients of the Eight Tested Components of the Mean Differences

Group	<i>N</i>	Mean	Variance	<i>m</i>	<i>d</i>	<i>h</i>	<i>i</i>	<i>j</i>	<i>l</i>	<i>dm</i>	<i>hm</i>
NZB	24	2.29	10.22	1	1	0	1	0	0	1	0
CBA/H	17	42.41	849.88	1	-1	0	1	0	0	-1	0
HNF <sub>1</sub>	31	90.16	721.14	1	0	1	0	0	1	1	0
NHF <sub>1</sub>	14	78.36	1022.40	1	0	1	0	0	1	-1	0
F <sub>2</sub>	21	47.43	2028.30	1	0	0.5	0	0	0.25	0	1
NH×H	25	35	920.25	1	0.5	0.5	0.25	0.25	0.25	1	0
H×HN	27	91	1614.60	1	0.5	0.5	0.25	0.25	0.25	0	1
HN×N	16	43.81	1341	1	-0.5	0.5	0.25	-0.25	0.25	-1	0
N×HN	32	49.19	1058	1	-0.5	0.5	0.25	-0.25	0.25	0	1

mother. The components of variation were analyzed with the COMPVAR program (Crusio, 1991). Four parameters were considered (Mather and Jinks, 1971): *D*, the additive component; *H*, the component due to dominance; *E*, due to the environmental effect of the genotype of the mother; and *S* (*dh*), which provides additional information on dominance. Males and females were pooled, as no sex differences were detected.

**Results and Discussion.** The sample sizes, mean untransformed values, and standard deviations for the strains and the derived crosses are shown in Table III. The coefficients for the eight components of the mean differences were derived from Mather and Jinks (1971) and are shown in Table III.

Three models fit the data: first, [*m*], [*d*], [*h*], [*i*], [*j*], [*dm*], [*hm*]; second, [*m*], [*d*], [*h*], [*i*], [*j*], [*l*], [*dm*]; and third, [*m*], [*d*], [*h*], [*i*], [*j*], [*hm*]. The three models differed for significant epistatic and maternal parameters, but epistatic and maternal effects were common to the three models. The additive and dominance effects [*d*] and [*h*] reached significance in the three models. The potence ratio, [*d*]/[*h*] (dominance/additivity) was always higher than 3, this high value indicating heterosis, as expected from the observation of the two F<sub>1</sub>'s. The F<sub>1</sub>'s did not indeed differ, but differed from *H*, the most emitting parental strain [*t*<sub>(44)</sub> = 4.60, *p* < 0.0002].

The *E* and *D* components of variation reached significance (*E* = 4.208 ± 1.226, *Z* = 3.431, *p* < 0.0003, and *D* = 4372.270 ± 2211.877, *Z* = 1.98, *p* < 0.02; one-tailed test in each case).

## GENERAL DISCUSSION

One day-old pups from seven highly inbred strains of laboratory mice were compared here with

regard to whistle and click emissions. The observed values fit very well with those obtained by Nitschke and Bell (1974) for the strains that were common to the two experiments, despite differences in recording conditions, involving their use of a colder temperature and older pups. In the two experiments, a drop in temperature reduced the number of whistles for C57BL/6J but not for BALB/cJ mice. The number of whistles during the 23°C condition was stable as shown by the comparison of seven strains that were common to both the present experiment and one which was published earlier (Cohen-Salmon *et al.*, 1985). The ranks are indicated after the name of each strain (in Roman numerals for the present experiment, Population I, and with those of the 1985 study indicated by italics), from the highest to the lowest emitter: C57BL/6J (1, 1), CBA/H (2, 2), BALB/cJ (3, 4), DBA/2J (4, 5), A/J (5, 3), XLII (6, 6), and NZB/BINJ (7, 7). The age function of whistles shown by Bell *et al.* (1972) was confirmed in experiment 2 and extended to clicks. The identical number of ultrasounds they found at 3 days of age in BALB/cJ and C57BL/6J mice, and the decrease they had observed for the latter strain, was replicated here. The number of whistles is thus a stable strain characteristic. Its suitability for genetic analysis is also enhanced by the presence of a clear-cut strain polymorphism, due mainly to the presence of two poorly emitting strains (XLII and NZB/BINJ) under both recording conditions.

The duration of whistles reported here is in agreement in the two populations and with previously published results (Hahn *et al.*, 1987; Bell *et al.*, 1972). The greatest contrast was observed for NZB/BINJ mice, which produced about six times as many shorter whistles than DBA/2J. Both XLII



and NZB/BINJ mice also contributed to the polymorphism of whistle frequencies.

The different method used to measure frequencies here and in previously published studies may be responsible for several discrepancies observed between our values and others, and not the low stability of these measures, since we obtained reasonably replicated results with Populations I and II. These differences are seen when the present values are compared to those published by Cohen-Salmon (1988). We analyzed the whole signal in the present study, whereas Cohen-Salmon selected small parts of the signal to be subject to spectral analysis.

The correlations estimated by the Hegmann and Possidente (1981) method are quite similar for the variables measured in Populations I and II. Those obtained in Population I show that there are at least two processes: one for each temperature condition. In the cold condition two clusters are deduced from the absence of overlapping between the range of correlations obtained for the duration of the whistles (Table II). The number of whistles have higher correlations with the frequencies ( $r_\alpha$  between 0.60 and 0.99) and lower with the durations (between 0.14 and 0.33).

Several sources of variation are involved in the number of whistles during the cold condition. Significant values of the effect of the mothers [ $dm$ ] or [ $hm$ ] according to the model and of environment  $E$  are observed in the analyses of the component of mean differences and of the variation.

In the analysis of the components of mean differences, the number of whistles is higher in the backcrossed populations derived from the parental strain mother than with the  $F_1$  mother. Several sources of variation are expected to contribute to this difference (Roubertoux *et al.*, 1990; Carlier *et al.*, 1990, 1992): X-chromosome, mitochondrial DNA, genomic imprinting, and maternal environments. An effect of the X chromosome might be discarded since we observed no sexual dimorphism for this variable. Mitochondrial DNA might be a candidate because it has different origins in NZB/BINJ and CBA/H (Yonekawa *et al.*, 1982), but this potential source of variation has to be eliminated because the congenic strains for mitochondrial DNA which had been developed previously (Carlier *et al.*, 1993) did not differ for the number of whistles recorded under identical conditions (Le Roy and Beau, in preparation). The contribution of

genomic imprinting or of maternal environments can thus be promoted but the genetic design used in the present experiment does not provide the opportunity to disentangle the potential respective implications of these two factors on number of whistles.

The heterotic inheritance and the significant epistatic components provide evidence for a polygenic or multigenic inheritance, in the populations derived from N and H.

A relatively high potence ratio indicates a directional dominance. This was present only in the analysis of the mean differences. Here, the high number of whistles is the dominant phenotype.

The additive component was significant for the components of both the variation ( $D$ ) and the means [ $d$ ]. The  $\alpha$  ratio was also significant with the Hegmann and Possidente (1981) method in the set of seven strains for the number of whistles. The significant values for [ $i$ ] and for [ $dm$ ] found in the crosses derived from N and H prevented us from ranking  $\alpha$  with  $V_a/V_p$  in the present case. The high value obtained for the  $\alpha$  ratio ( $0.687 \pm 0.162$ ) could not lead us to rule out the contribution of [ $d$ ] to the number of whistles in the cold condition and, consequently, to assume an adaptive value for this trait. A significant additive component was not observed by Hahn *et al.* (1987) for the same variable at 5 days of age. This could be due to the small difference between BALB/cJ and DBA/2J mice and to the fluctuating values observed for these strains between day 4 and day 6.

An adaptive value would be expected if ultrasounds were the exclusive trigger of maternal behavior and, thus, the exclusive target for the selection. Rosenblatt (1967), and Rosenblatt *et al.* (1979) pointed out the complexity of the female's responses toward her progeny and described the multichannel sensorial process implicated in the maintenance of maternal behavior. Beach and Jaynes (1956) and Smotherman *et al.* (1974) demonstrated that vocalization's role in retrieving is potentiated by the other sensorial cues. An alternative hypothesis suggests that behavioral traits are not only the target of evolutionary processes *via* selection but also a tool for evolution (Roubertoux, 1981). In this case, they contribute to the maintenance of genetic variation or protect genetic diversity against selection. Vocalization in newborn mice could play this role. It has polygenic correlates, and moreover, dominance and epistasis con-

tributes to its variation. Finally, there is no coselection for the ability of the females to perceive, and the ability of the pups to produce, the acoustic signals: mothers are unable to discriminate the auditory cues produced by their own pups in several strains of laboratory mice (Ralls, 1967; Cohen-Salmon *et al.*, 1985). These criteria characterize a trait (Roubertoux, 1981, 1993) that may contribute to the maintenance of genetic variation in a species.

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