

Male Polymorphism in *Limia perugiae* (Pisces: Poeciliidae)

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The male-polymorphic poeciliid fish, *Limia perugiae*, a small teleostean endemic to the southeast of the Caribbean island Hispaniola, consists of three male size morphs with uniform females. Large males differentiate at a size varying between 25 and 38 mm; intermediate males, between 21 and 25 mm. Under competition, large males exhibit an elaborate courtship display, whereas small males show only a sneak-chase behavior. Intermediate males adapt their tactics to the respective competitors. However, all male morphs can switch from courtship display to sneak-chase behavior. In large mating groups with four males of different size and five or six virgin females, large dominant α -males as well as small subordinate δ -males did not produce any offspring. Unexpectedly, all progeny were sired exclusively by the intermediate subordinate β - and γ -males. Breeding experiments with the three male morphs can best be explained by a model of Y-linked genes for small and large size which are both suspended by the activity of an autosomal recessive repressor responsible for the development of intermediate males. The dominant allele of the recessive repressor, in either its homozygous state, activates the Y-chromosomal genes for large or small size, respectively. Accordingly, intermediate males may produce male offspring of all size classes, depending on the presence of either the Y-linked gene or the autosomal repressor.

KEY WORDS: Poeciliid fish; male size polymorphism; reproductive success.

INTRODUCTION

Recently, several instances of male size polymorphism have become known in poeciliid fishes. Thus, in two species of pygmy swordtails, *Xiphophorus nigrensis* and *X. multilineatus*, large and small males exhibit different mating strategies (Kallman, 1984; Ryan, 1988, 1993; Zimmerer and Kallman, 1988, 1989). Differences in adult male size and age at

sexual maturity of *X. multilineatus* (Rauschenberger *et al.*, 1990) are controlled by genetic variation at a Y-linked locus which determines four genetic size classes. The mating behavior of the males of the three largest size classes consists exclusively of an elaborate courtship display, whereas that of the genetically small males ranges from display to a sneak-chase behavior. As expected, females prefer the display of large males. In mating-competition experiments (two females with one large male and one small male), the large males are dominant and deny the small males' access to females. From 20 such experiments, 601 large-male and 200 small-male progeny were obtained, indicating that the switch to sneak-chase behavior by small males is not particularly effective in overcoming the large-male advantage. By using the largest males of the genetically smallest size class and the smallest males

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of the genetically next-larger size class, size was kept constant, whereas genotype was varied. When these males were tested in competition with genetically large males, only the males of the genetically smallest size class showed sneak-chase behavior. These observations suggest that the difference in mating behavior is not an indirect developmental effect of size but, rather, is under genetic control.

The present paper reports mating strategies and genetic determination of three male size morphs in *Limia perugiae*. Like most poeciliid species, these different male morphs differentiate into males after a protogynic developmental passage. At sexual differentiation, all males cease their growth because of the activity of testosterone, whereas the uniform females continue to grow also after reaching sexual maturity. As in the pygmy swordtail, large males of *L. perugiae* also show an elaborate courtship display, while that of small and intermediate males ranges from courtship to sneak-chase behavior.

MATERIALS AND METHODS

Maintenance and Origin of the Fish. The fish are kept in aquaria of 8-, 25-, 50-, 100-, or 200-L volume at a temperature varying between 25 and 28°C. They were fed on Teramin, nauplia of *Artemia salina*, and *Daphnia* species. The artificial illumination by neon lights was set to a 12-h rhythm (0700–1900 h), but daylight was not excluded. All tanks were filled with Munich tap water (pH 7.5) and were artificially aerated. Only observation tanks were supplied with gravel.

Every morning all basins containing mated pairs or pregnant females were routinely checked for newborn fry or dead fish. Because the broods were sometimes born over a period of several hours and because some females might have been cannibalistic, all fry were immediately removed to small glass vessels and later transferred to rearing aquaria. Aquatic plants, *Fontinalis* spp. and *Cryptocoryne* spp., were grown in aquaria in which broods were anticipated to enable newborn fish to hide in the vegetation.

To obtain virgin females which are receptive to male sexual activities, differentiating females were separated from their male sibs at the time when the males' anal fin began to transform into a gonopodium, the male copulatory organ.

All fish used in these experiments were originally obtained from Mr. Manfred K. Meyer, Bad Nauheim, Germany, who collected them either in

a small river near Piedra Blanca, in the Dominican Republic, or in its Western karst headwaters. Since 1978, populations with large and small males have been kept separately in large collective breedings in our laboratory.

Scoring Behavioral Activities. With the exception of the competition experiments in 160-L tanks which were equipped with high-grown water plants (*Hygrophila* spp.), all experiments were carried out for 20 min in 37.5-L tanks with water moss (*Fontinalis* spp.) by direct observation. Social behavior activities were scored and analyzed with an event recorder system (EV.24), the hard- and software of which was specially manufactured and equipped by Ingenieurbureau Erbacher (Buchenrain nr. Munich, Germany).

Male Sexual Behavior Activities. After recognizing a receptive female, the male follows her and begins to nibble her genital pore by repeatedly touching it with his nose. The male then tries to corner the fleeing female in order to present himself in front of her with all fins spread and the vertebral column sigmoidally curved (*display* behavior). Mostly after various sequences of following, displaying, and nibbling, the male attempts to copulate by orienting behind the female, swimming forward, slanting his body, and bringing his gonopodium into a forward position at an angle of more than 90° toward the female's genital pore, thereby attempting to insert his gonopodial tip into the female's gonopore (*gonopodial thrusting*). Because all poeciliid females store spermatophores in their oviduct giving rise to more than ten successive broods without any new insemination, copulation attempts normally occur more often than copulations. True copulations are recognizable in all poeciliids by the postcopulation jerking movements of the male.

Male Agonistic Behavior. Under competition, large dominating males attack other males, apparently in order to exclude all competitors from access to receptive females. Because a linear rank order will be established soon after several males are put together, no serious lesions normally appear.

Female Choice Behavior. A 37.5-L aquarium was subdivided by a pane of glass into three equal compartments. Every six virgin test females had to choose between one large male on one side of their compartment and one small male on the other side. Accordingly, the test females were located in the middle compartment. The frequency at and for which duration the females touched the pane separating

their compartment from those of the two competing males were recorded. To avoid the effect of possible side preferences of the test females, the two males had to exchange positions after 20 min of observation, i.e., they were removed from the compartment in which they spent the first 20 min and put in the opposite compartment for the second 20 min of observation. Thus, the choice behavior of the six females was observed for 40 min.

Statistical Treatment. After analyzing all experimental data for normal distribution by the Kolmogorov-Smirnov test, parametric and nonparametric methods were used with the aid of STATGRAPHICS (Version 2.1, Serial Number 282827, Statistical Graphics Corp.) and the test procedures given by Siegel (1956) and Sachs (1973).

RESULTS

Onset of Sexual Differentiation. While no significant growth difference in females of different origins was observed, the growth differences found in males depend not on age but on size. Small males differentiate at a size of < 21 mm. They reach a total length of 25.5 mm (measured with an accuracy of ± 0.5 mm) at an age of between 8 and 14 weeks. Their growth ceases and accordingly the growth curve flattens out from the 20th week after birth. Intermediate males differentiate at a size of between 21 and < 25 mm, whereas large males differentiate at a size of ≥ 25 mm between 8 and 18 weeks of age. The large males' growth curve flattened out at an age of 24 weeks and at a maximal total length of 31 mm. As in other poeciliid species, growth speed depends both on temperature and water volume available per growing fish.

Male Mating Strategies. In *Limia perugiae*, large and small males exhibit alternative mating strategies: While large males court females in a conspicuous manner (Fig. 1), exhibiting a courtship display with all fins spread in front of the female, small males normally follow the females, nibbling, and finally, attempt to inseminate them with gonopodial thrusts, often without the female's acceptance. Accordingly, small males exhibit a lower display rate than large males (Fig. 1), while the small male's number of copulation attempts by gonopodial thrusting exceeds that of large males (Fig. 2). Thus, gonopodial thrusting is the most prominent sexual behavior of "sneaking" small males. There are also morphological differences: The gonopodial length of small males in relation to their

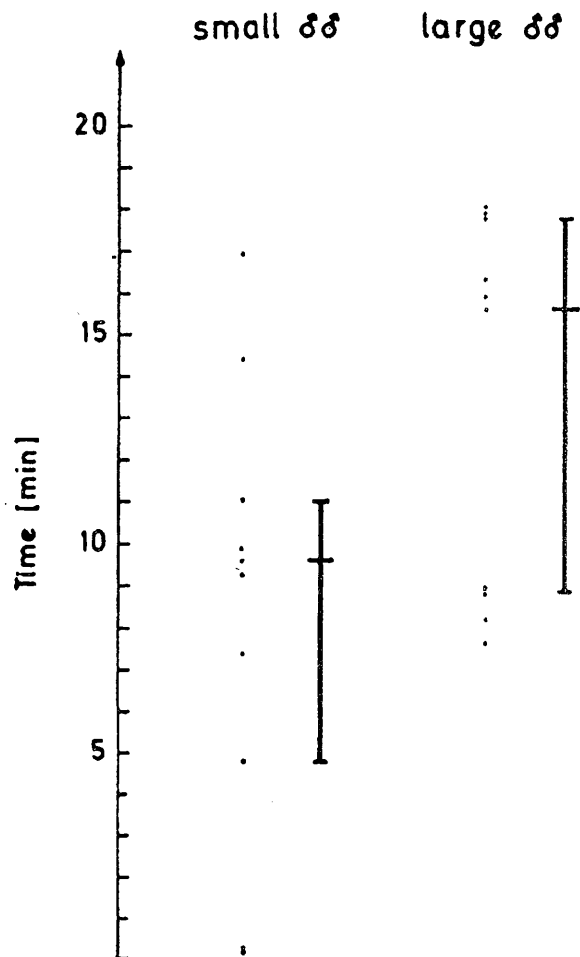


Fig. 1. In one-male/one-female matings, large males ($n = 10$) spend more time displaying for females than small males ($n = 10$) ($U = 23, p < .05$).

standard length (= total length minus length of the caudal fin) is greater than that of large males (Fig. 3). To prevent the loss of pursued females in the density of the vegetation, small males perform *contact nibbling*, i.e., they attempt to maintain contact with the female's genital pore by continuously touching it with their mouth. Large males display mainly *identification nibbling* by probing to see whether or not the female is in the receptive phase of her ovarian cycle. This nibbling behavior (Fig. 4) is related to size and seems therefore to be inherited together with size. When large males dominate other males in order to gain exclusive access to females, they spend more time attacking large

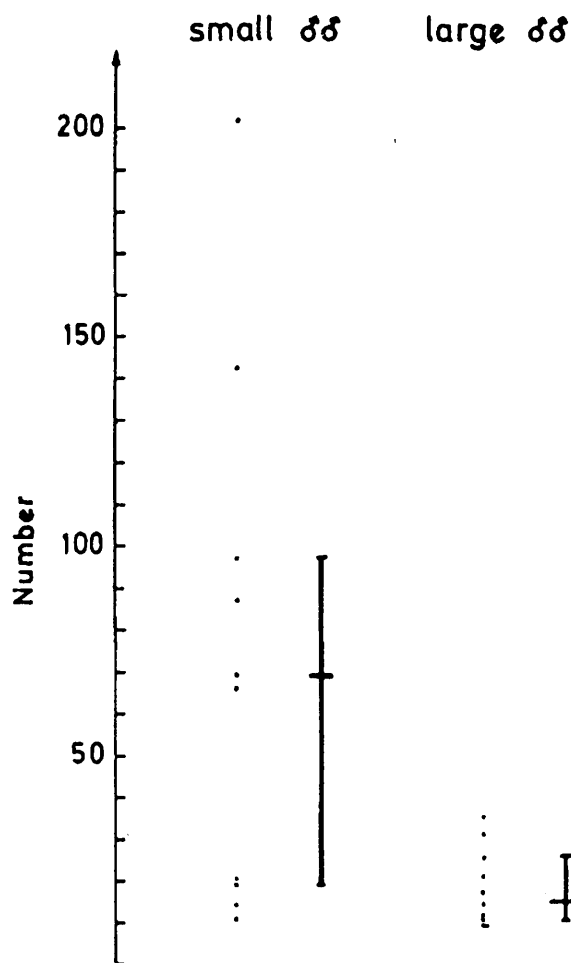


Fig. 2. In one-male/one-female matings, small males ($n=10$) deliver more gonopodial thrusts (= copulation attempts) than large males ($n=10$) ($U=14$, $p < .02$).

males which approach females than attacking small males.

Female Choice. Independent of males' social behavior, females prefer large over small males (Fig. 5). Only in 1 of 18 replicates did the females prefer a small male to a large one.

Significance Level. All behavioral differences found between large and small males were statistically significant [chi-square method and Mann-Whitney U test according to Siegel (1956); $p < .05$]. Individual scores and group medians with quartiles are given in Figs. 1-5.

Genetic Determination of Male Size. The overall segregation ratio of 268 males to 269 females

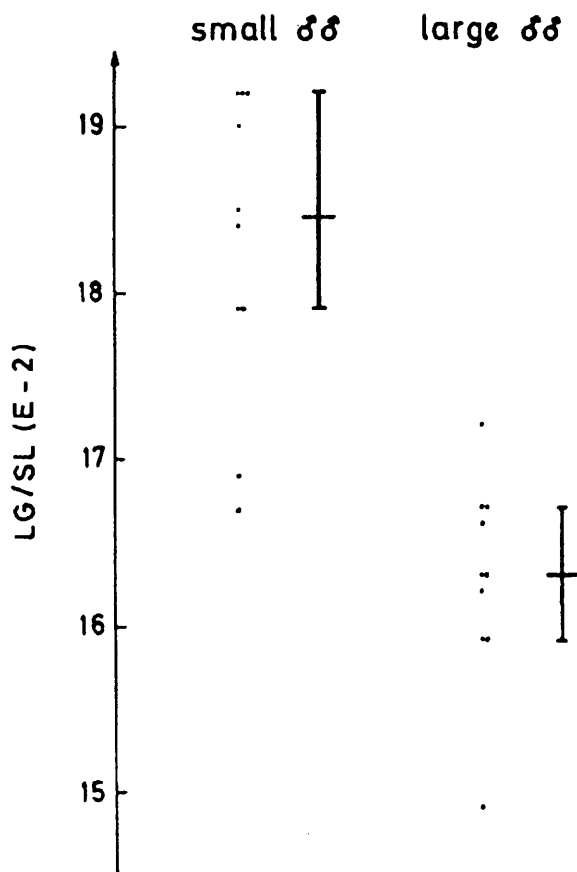


Fig. 3. Small males' gonopodium length in relation to their standard length is larger than that of large males ($n=10/10$; $U=4$, $p < .001$).

($\chi^2 = .00186$, $p < .965$) among the progeny of defined *L. perugiae* broods favors the view of a gonosomal sex-determination mechanism in that species (Kallman, 1984). All segregation ratios among male offspring after mating fish of known pedigree (Table I) can be explained most parsimoniously by the assumption that the development of large and small males is mediated by the activity of two Y-chromosomal genes, *Large* (L) and *Small* (S). Intermediate males also possess either a Y-Large (Y_L) or a Y-Small (Y_S) chromosome, the effectiveness of which is suspended by the Activity of an autosomal recessive repressor, r/r , while its dominant allele, in either the homozygous (R/R) or the heterozygous (R/r) state, activates the respective Y-chromosomal gene L or S (Table II). Depending on the autosomal constitution of the X/X

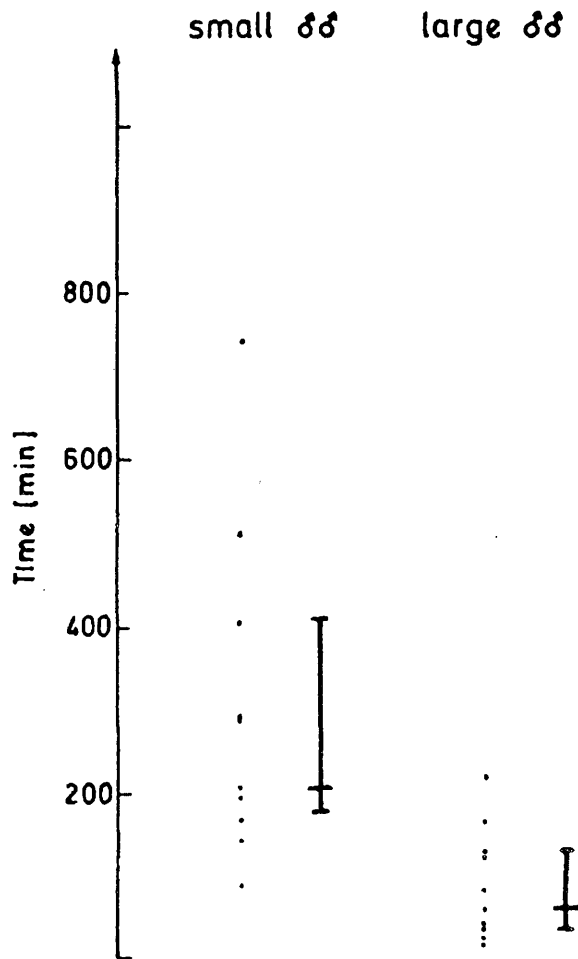


Fig. 4. In one-male/one-female matings, small males ($n=10$) spend more time nibbling than large males ($n=10$) ($U=9$, $p < .002$).

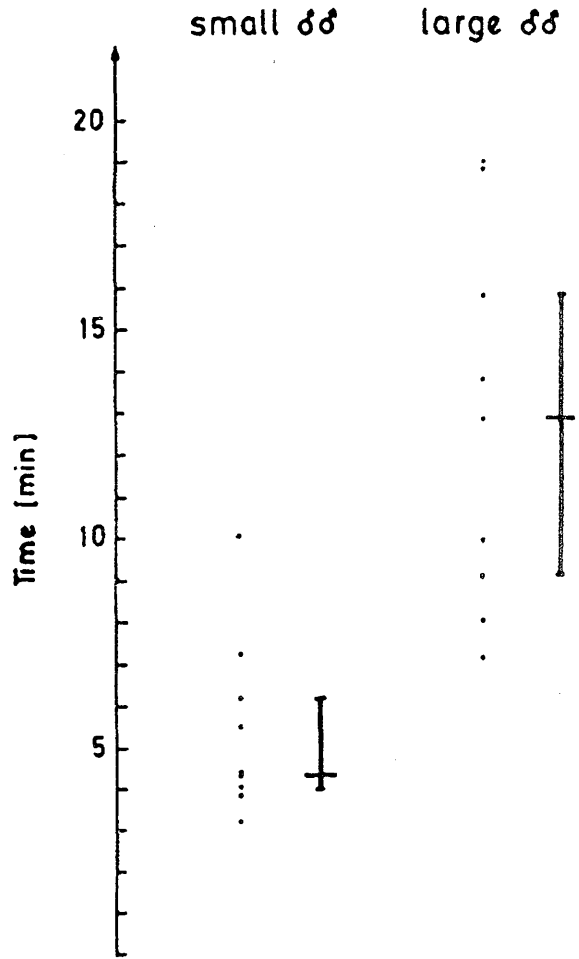


Fig. 5. Females spend more time near large males than near small males (randomization test: $n=9$; $d_1=2$; $p < .004$).

females, which also can be either homo- or heterozygous for the two repressor alleles R and r , all size classes can reappear in the progeny of intermediate males. Since recombinations between X and Y chromosomes are not known in *L. perugiae*, they probably will not influence the genetic determination of male size polymorphism in that species as proposed in Table II. Other models such as inheritance via an X-linked gene complex acting primarily through the pituitary gland, as hypothesized for the inheritance of different male size in *Xiphophorus* (Bao and Kallman, 1982; Kallman and Borkoski, 1978), would not fit the segregation ratio of 25% intermediate to 75% large male offspring as found in one cross (Table I).

DISCUSSION

The cooperation of Y-chromosomal genes for either large or small size with an autosomal recessive repressor, as proposed here for *L. perugiae*, does not agree with previous explanations given for male size inheritance in the pygmy swordtail, *X. multilineatus* (Zimmerer and Kallman, 1988, 1989). In the latter peociliid, all X chromosomes possess an allele for early maturation at a small size, whereas the Y chromosomes may carry one of four P alleles controlling the onset of sexual maturity at different sizes in males. In competition experiments, only large males competed with small males; large males then produced three times as many offspring as did small males. The mating success in large mating

Table I. Inheritance of Polymorphic Phenotypes in Male *Limia perugiae*

Parental cross	Male offspring			Segregation ^a
	Small (S)	Inter-mediate (I)	Large (L)	
Small male (Y _S , R/r × female (r/r)	17	17	0	50% S:50% I
Small male (Y _S , R/R or (R/r) × female (R/r or R/R)	10	0	0	100% S
Intermediate male Y _L , r/r; from large father) × female (R/r)	0	31	18	50% I:50% L
Large male (Y _L , R/R) × female (r/r)	0	34	37	50% I:50% L
Large male (Y _L , R/R) × female (R/r)	0	10	42	25% I:75% L

^a All segregation ratios obtained among male offspring do not deviate significantly from the theoretical segregation ratio (standard chi-square method, $p > .05$).

Table II. Genetic Explanation of Size Polymorphism in Male *Limia perugiae*

Autosomal constitution	Y chromosome	
	Y _L	Y _S
R/R or R/r	Large	Small
r/r	Intermediate	

groups of *X. multilineatus* in which all four size classes of males competed with each other has not yet been determined. The reproductive success of large male pygmy swordtails competing with small ones agrees sufficiently with the reproductive success of large *L. perugiae* males competing only with small males: Studies on the reproductive success of one large dominant male competing with one small subordinate male revealed a mating success of 100% for the large male. Most recently, however, we determined the paternity of large, intermediate, and small males by DNA fingerprinting of their offspring (for methods see Nanda *et al.*,

1990) also in large mating groups with one dominant large α -male, two intermediate β - and γ -males, and one subordinate δ -male competing for five or six virgin females (Table III). Under these conditions, however, the large male's contribution to the offspring unexpectedly dropped to zero (Schartl *et al.*, 1991). This result was obtained despite the fact that the large α -male dominated all three other males. He delivered twice as many attacks as did all three subordinate males. Also, the α -male courted the virgin females much more than its competitors did (Table IV). All progeny were sired exclusively by the intermediate-sized β - and γ -males. Because these males carry either a Y_S or a Y_L chromosome, large and small males can be recruited in their progeny.

Since the agonistic behavior of the large, dominant male apparently did not exclude competitors from access to females, the question of the biological meaning of aggressiveness in this species remains open.

Table III. Paternity of F₁ Offspring ($n = 106$) of Large Mating Groups of *Limia perugiae* (According to Schartl *et al.*, 1991)

Females	Males according to rank order				
	α	β	γ	δ	Uncertain
Expt 1					
1	0	6	0	0	0
2	0	10	0	0	0
3	0	4	0	0	1 ^a
4	0	8	0	0	0
Uncertain	0	1	0	0	0
Total	0	29	0	0	1
Expt 2					
1	0	0	1	0	0
2	0	7	1	0	4 ^b
3	0	15	0	0	1 ^c
4	0	0	1	0	0
5	0	25	6	0	3
n.d. ^d	0	6	6	0	0
Total	0	53	15	0	8

^a Not unequivocally ascribable to one of the four possible fathers.

^b Attributable to β -male.

^c By assumption of one mutation, attributable to β -male.

^d Mother not determined. The similarity of the fingerprint pattern of females (high inbreeding coefficient) in the second experiment precluded unequivocal determination of maternity in some cases.

Table IV. Quantitative Analysis of Male Social Behavior in Large Mating Groups of *Limia perugiae* (According to Scharl *et al.*, 1991^a)

Male social behavior ^b	Rank order of four males			
	α	β	γ	δ
Courtship display	1.44	.11	.04	0
D	(.83, 7.43)	(0, .58)	(0, .47)	
F	14	2	2	0
	(7, 21)	(0, 8)	(0, 6)	
Following	1.06	.15	.8	.94
D	(.63, 1.45)	(.02, .94)	(.31, 1.85)	(.17, 2.71)
F	21	3	10	12
	(15, 26)	(1, 15)	(7, 21)	(7, 12)
Nibbling	.31	.04	.15	.15
D	(.22, .47)	(0, .19)	(.06, .4)	(.03, .4)
F	6	3	3	4
	(5, 9)	(0, 5)	(1, 8)	(1, 7)
Copulation attempts	2	1	6	7
F	(0, 4)	(0, 4)	(2, 16)	(3, 13)
Agonistic attacks	2.36	.03	.16	0
D	(.73, 3.26)	(0, .25)	(.05, .35)	
F	34	n.d. ^c	4	0
	(10, 40)		(2, 8)	

^a Values are means (minimum, maximum).

^b D, duration (minutes); F, frequency (number of actions).

^c Not determined.

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