method and suggests that general acid-base catalysis by nucleotide side chains is a mechanism that can be used in RNA-catalyzed reactions.

References and Notes

1. T. R. Cech and B. L. Golden, in *The RNA World*, R. F. Gestland, T. R. Cech, J. F. Atkins, Eds. (Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, ed. 2, 1999), pp. 321–349.
2. G. J. Narlikar and D. Herschlag, *Annu. Rev. Biochem.* **66**, 19 (1997).
3. C. Walsh, *Enzymatic Reaction Mechanisms* (Freeman, New York, 1979).
4. W. Saenger, *Principles of Nucleic Acid Structure*, C. R. Cantor, Ed. (Springer Advanced Texts in Chemistry, Springer-Verlag, New York, 1984).
5. P. Legault and A. Pardi, *J. Am. Chem. Soc.* **116**, 8390 (1994); G. J. Connell and M. Y. Yarus, *Science* **264**, 1137 (1994).
6. A. T. Perrotta and M. D. Been, *Nature* **350**, 434 (1991).
7. T. S. Wadkins, A. T. Perrotta, A. R. Ferré-D'Amaré, J. A. Doudna, M. D. Been, *RNA* **5**, 720 (1999).
8. A. T. Perrotta and M. D. Been, *Biochemistry* **31**, 16 (1992).
9. H.-N. Wu *et al., Proc. Natl. Acad. Sci. U.S.A.* **86**, 1831 (1989); M. Y.-P. Kuo, L. Sharmeen, G. Dinter-Gottleib, J. Taylor, *J. Virol.* **62**, 4439 (1988).
10. A. R. Ferré-D'Amaré, K. Zhou, J. A. Doudna, *Nature* **395**, 567 (1998).
11. In both ribozymes, numbering is from the cleavage site so that cleavage occurs between positions −1 and 1. Because of differences in the sequences of the two ribozymes, we use a γ prefix to denote genomic nucleotide positions. γC75 and C76 were first identified by mutagenesis as essential for cleavage activity (*13, 22, 23*) . Changing γC75 to U, G, or A resulted in no detectable self-cleavage activity in the genomic ribozyme; these mutations were reported to decrease activity by a factor of at least 5×10^5 (*23*). In the antigenomic ribozyme, changing C76 to U or G results in little or no activity (decreased by a factor of $>10^4$), whereas the C76 to A mutant is marginally active (decreased by a factor of 2×10^3) (*13*). Because the published data for the C-to-A mutations in the two ribozymes conflicted, we tested the genomic γC75a mutation. It cleaved with a rate constant of 3.7×10^{-3} min^{-1}, down a factor of 5×10^3 relative to the wild-type sequence—essentially the same effect as in the antigenomic ribozyme (T. S. Wadkins and M. D. Been, unpublished data). Cross-linking data indicate that C76 is within 10 Å of the cleavage-site phosphate [C. Bravo, F. Lescure, P. Laugâa, J.-L. Fourrey, A. Favre, *Nucleic Acids Res.* **24**, 1351 (1996); S. P. Rosenstein and M. D. Been, *Biochemistry* **35**, 11403 (1996)]. Solid evidence for γC75 being part of the active site of the genomic ribozyme was seen with the crystal structure of the 3′ cleavage product: the O-2 of γC75 could form a hydrogen bond to the 5′-hydroxyl leaving group (*10*) . Ferré-D'Amaré *et al.* (*10*) noted that the base of γC75 is within a region of negative electrostatic potential, with its N-4 amino group participating in a network of hydrogen bonds. They proposed that this environment may perturb the pK_a of the base and that, in the precursor, the N-3 of γC75 may be positioned to accept the proton from the 2′-hydroxyl group of the ribose at position −1.
12. The antigenomic ribozymes used in this study are derivatives of PEX1 [A. T. Perrotta and M. D. Been, *J. Mol. Biol.* **279**, 361 (1998)] and were prepared by transcription from restriction endonuclease–cut plasmid DNA with T7 RNA polymerase. Precursor RNA was purified by electrophoresis on polyacrylamide gels and eluted. The precursor RNAs were either labeled internally by including guanosine [α-^{32}P]triphosphate in the transcription mixture or 5′-end-labeled with adenosine [γ-^{32}P]triphosphate and polynucleotide kinase after dephosphorylating with calf intestinal phosphatase (*6*). The mutations at position 76 and modifications to the sequence 5′ to the cleavage site were generated by oligonucleotide-directed mutagenesis on single-stranded uracil-containing templates of the plasmid (*6*).
13. A. T. Perrotta and M. D. Been, *Nucleic Acids Res.* **24**, 1314 (1996).
14. The rate constant for cleavage of the C76u ribozyme increased with increasing cytosine concentration; at 40 mM cytosine, there was a 57-fold stimulation ($k_{obs} = 8.5 \times 10^{-4}$ min^{-1}). Buffers that were tested and failed to support activity were tris-HCl, Hepes, Pipes, and MES at pH 7.5; CHES at pH 8.5; and sodium acetate at a variety of pH values. Other potential bases—ethylenediamine (pH 7.8), pyridine (pH 7.5), and ammonia (ammonium acetate, pH 8.5)—failed to rescue cleavage activity. The tris buffer did not inhibit imidazole-dependent cleavage of the C76u ribozyme. However, significant stimulation by imidazole was not a general phenomenon for the HDV antigenomic ribozyme. With the wild-type ribozyme, a moderate increase in the rate constant (1.2-fold) occurred with the addition of 200 mM imidazole (pH 7.4) to the reaction mixture (Table 1). We observed no stimulation of cleavage activity with the C76a ribozyme.
15. A. Peracchi, L. Beigelman, N. Usman, D. Herschlag, *Proc. Natl. Acad. Sci. U.S.A.* **93**, 11522 (1996); A. Peracchi, J. Matulic-Adamic, S. Wang, L. Beigelman, D. Herschlag, *RNA* **4**, 1332 (1998).
16. A. T. Perrotta, I.-h. Shih, M. D. Been, data not shown.
17. A. Jack, J. E. Ladner, D. Rhodes, R. S. Brown, A. Klug, *J. Mol. Biol.* **111**, 315 (1977).
18. D. Michalowski, J. Wrzesinski, W. Kryzosiak, *Biochemistry* **35**, 10727 (1996).
19. E. Anslyn and R. Breslow, *J. Am. Chem. Soc.* **111**, 4473 (1989); A. Roth and R. B. Breaker, *Proc. Natl. Acad. Sci. U.S.A.* **95**, 6027 (1998).
20. S. Huang and S. C. Tu, *Biochemistry* **36**, 14609 (1997).
21. S. L. Newmyer and P. R. Ortiz de Motellano, *J. Biol. Chem.* **271**, 14891 (1996).
22. P. K. R. Kumar *et al., Nucleic Acids Res.* **20**, 3919 (1992).
23. N. K. Tanner *et al., Curr. Biol.* **4**, 488 (1994).
24. D. D. Perrin and B. Dempsey, *Buffers for pH and Metal Ion Control* (Chapman & Hall, London, 1974).

25. Supported by NIH grant GM47322. We thank T. Cech, S. Crary, C. Fierke, D. Herschlag, T.-s. Hsieh, and T. Wadkins for critical comments and helpful suggestions.

18 June 1999; accepted 6 August 1999

Male Attractiveness and Differential Testosterone Investment in Zebra Finch Eggs

Diego Gil,*† Jeff Graves, Neil Hazon, Alan Wells

Good-genes hypotheses of sexual selection predict that offspring fathered by preferred males should have increased viability resulting from superior genetic quality. Several studies of birds have reported findings consistent with this prediction, but maternal effects are an important confounding variable. Those studies that have attempted to control for maternal effects have only considered differential maternal investment after egg laying. However, female birds differentially deposit testosterone in the eggs, and this influences the development of the chick. This study shows that female birds deposit higher amounts of testosterone and 5α-dihydrotestosterone in their eggs when mated to more attractive males.

Female preferences for ornaments that indicate male genetic quality would allow females to enhance the viability of their offspring (*1*). Evidence of female preference for traits indicative of "good genes" has been found in some species (*2*). In birds, several studies have found evidence for enhanced survival of offspring fathered by highly ornamented males (*3*, *4*), but it remains possible that this effect is a result of differential female investment in the offspring of these males. Differential investment by females in chicks fathered by attractive males has been experimentally demonstrated in lab and field studies (*5*).

Although some of the studies reporting good-genes effects in birds have attempted to control for maternal effects, these studies have only considered investment after laying (*4*). However, it is possible that differential investment may occur before laying. Females of several bird species deposit varying amounts of testosterone in their eggs (*6–9*), and this variation influences the development of the chick. Chicks that hatch from eggs with high amounts of testosterone beg for food more intensively, grow faster than other chicks, and are more likely to become dominant once they fledge (*6*, *10*).

We predicted that female birds would deposit higher amounts of testosterone when mated to attractive males than when mated to less attractive males. In the zebra finch (*Taeniopygia guttata*), the attractiveness of the male can easily be manipulated by using leg bands of different colors. Females pair preferentially with red-banded males and avoid green-banded ones. This manipulation has a more substantial effect on male attractiveness than any other

School of Environmental and Evolutionary Biology, University of St. Andrews, St. Andrews, Fife KY16 9TS, UK.

*Present address: Laboratoire de Psychophysiologie et d'Ethologie, UPRESA-CNRS 7025 Université de Paris X, 92001 Nanterre, France.

†To whom correspondence should be addressed: E-mail: Diego.Gil@u-paris10.fr.

measured male characteristic (*11*).

We conducted an experiment in which male zebra finches were given either red or green color bands on both legs. Males were randomly assigned to either color band. Twelve females were randomly allocated to two groups of six, each with either a red- or green-banded male for their first mate. Eggs were removed for hormone analysis after laying and replaced by dummy eggs. After completion of the clutch, the nest was removed, and all females were given a different mate with the other color band and allowed to lay a second clutch (*12*).

The amount of testosterone (T) and 5α-dihydrotestosterone (DHT) in the yolk was measured together first, and then DHT alone was measured. The amount of T was calculated from these two measurements (*13*).

Females mated with red-banded males deposited significantly more T and DHT in the eggs than did the same females when mated with green-banded males (Fig. 1). This finding questions the validity of the existing evidence for good-genes models of sexual selection in birds. Because experimental manipulation of yolk testosterone concentrations in canaries (*Serinus canaria*) increases both growth rates and begging rates (*10*) and there is a correlation between the concentration of yolk testosterone and the social rank when fledged (*6*), the increased viability of the chicks could be due to females investing higher concentrations of androgens in the eggs after mating with more attractive males. Our results show that it is essential to control for this form of differential maternal investment before concluding that females are getting good genes for their offspring by mating with more attractive males.

Why do females not put the same amount of testosterone in their eggs, regardless of male attractiveness? The variation in testosterone investment between clutches suggests that this investment has a cost, incurred either by the females or the offspring. The concentration of testosterone in the yolk of the egg is positively correlated with the amount of testosterone in the female during the yolk phase (*7*). Testosterone concentrations in females increase with high rates of intrasexual aggression (*14*), but little is known about the effects of increased concentrations in females (*15*). If male attractiveness correlates with offspring viability (*3–4*), then it might pay females to incur a cost and to invest more testosterone in the offspring fathered by attractive males. This would be the case whether the benefits to the female were direct or indirect (genetic).

Alternatively, the cost might be borne by the offspring. If increased concentrations of testosterone in the yolk suppress the immune system of the developing chick (*16*), a good-genes theory would predict that only chicks sired by highly ornamented males would be able to withstand high concentrations of testosterone. There is evidence that physiological concentrations of testosterone depress immune function when administered to young chickens (*17*), although in adults the evidence is mixed (*18*). We do not know if exposure to high concentrations of testosterone in the egg suppresses the immune system in the developing chick, but if so, the pattern of differential investment of testosterone between clutches that we have found could be an adaptive response; chicks fathered by less ornamented males would not normally be able to withstand the stress of higher amounts of testosterone.

Hatching asynchrony is a widespread phenomenon in birds in which the last eggs of a brood hatch later than the first (*19*). This hatching asynchrony results in a feeding hierarchy within the brood that is a good predictor of which chick will die in the case of brood reduction. The uneven distribution of testosterone within a clutch has important repercussions for the chicks that hatch last; chicks with extra testosterone will be given a

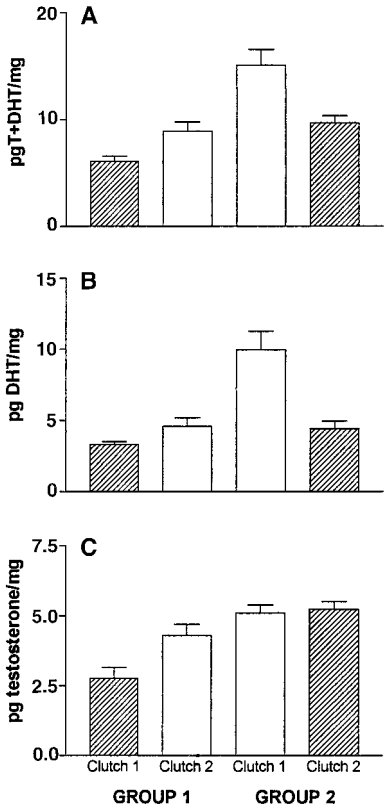

Fig. 1. Mean contents per milligram of yolk of (**A**) testosterone (T) plus 5α-dihydrotestosterone (DHT), (**B**) DHT, and (**C**) T (error bars show standard errors). The open bars represent the condition when the females were mated to red-banded males, and the shaded bars represent the condition when the females were mated to green-banded males. Group 1 females were mated to a green-banded male first and then to a red-banded male. Group 2 females were mated to a red-banded male first and then to a green-banded one. Analysis of variance (ANOVA) showed that females with red-banded mates laid eggs with significantly higher concentrations of all three androgen measures than did the same females when mated to green-banded males [repeated measures ANOVA with females nested within order and clutches within females: T + DHT: $F(1,52) = 47.8$, $P < 0.0001$; DHT: $F(1,52) = 19.4$, $P = 0.001$; T: $F(1,52) = 5.46$, $P = 0.023$]. The interaction between order and treatment was also significant for all three [T + DHT: $F(1,52) = 26.97$, $P < 0.0001$; DHT: $F(1,52) = 18.80$, $P = 0.0001$; T: $F(1,52) = 23.55$, $P < 0.001$].

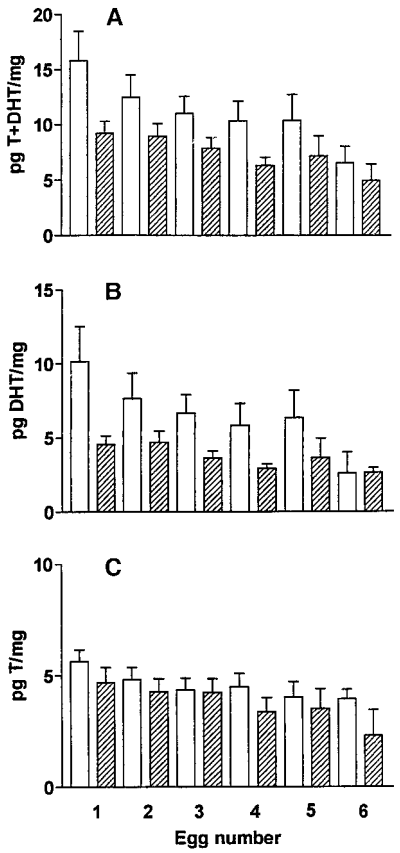

Fig. 2. Mean contents per milligram of yolk of (**A**) T plus DHT, (**B**) DHT, and (**C**) T for position in the laying order (error bars show standard errors). The open bars represent the condition when females had a red-banded mate, and the shaded bars represent the condition when the mate was green-banded. An analysis of covariance found that there was no difference in the slopes for females with red- or green-banded mates [T + DHT: $F(1,107) = 1.11$, $P = 0.29$; DHT: $F(1,107) = 1.98$, $P = 0.16$; T: $F(1,107) = 0.04$, $P = 0.85$]. All regressions were significantly negative: T + DHT for red-banded males: $y = 16.3 - 1.48x$, $P = 0.017$; T + DHT for green-banded males: $y = 10.2 - 0.78x$, $P = 0.016$; DHT for red-banded males: $y = 10.6 - 1.14x$, $P = 0.027$; DHT for green-banded males: $y = 5.02 - 0.39x$, $P = 0.045$; T for red-banded males: $y = 5.68 - 0.34x$, $P = 0.038$; T for green-banded males: $y = 5.14 - 0.39x$, $P = 0.043$.

head start in sibling rivalry (*8*). Both increasing and decreasing amounts of testosterone with laying order of eggs have been described in different species (*6*, *8*, *9*). This has been interpreted as maternal manipulation to ameliorate or impair, respectively, the survival prospects of the last-hatched chicks. Zebra finches do show asynchronous hatching, but it is more pronounced in the lab than in the field (*20*). The amount of T and DHT that female zebra finches deposited in the egg declined with the position in the laying sequence, regardless of males' leg-band color (Fig. 2), suggesting that females are reinforcing the feeding hierarchy within the brood. This could be adaptive when food conditions are poor or very unpredictable. If the probability of brood reduction covaried with male quality—that is, if brood reduction were less likely when females were paired to attractive males—then we would expect that testosterone would be more evenly distributed within the clutches fathered by red-banded males than in those fathered by green-banded males. However, the regression slopes of androgens with egg laying order were not different between the two conditions (Fig. 2), suggesting that the conditions leading to adaptive brood reduction in this species are more dependent on environmental conditions than on male quality.

References and Notes

1. M. Andersson, *Sexual Selection* (Princeton Univ. Press, Princeton, NJ, 1994).
2. A. M. Welch, R. D. Semlitsch, H. C. Gerhardt, *Science* **280**, 1928 (1998); G. S. Wilkinson, D. C. Presgraves, L. Crymes, *Nature* **391**, 276 (1998); A. Hoikkala, J. Aspi, L. Suvanto, *Proc. R. Soc. London Ser. B Biol. Sci.* **265**, 503 (1998).
3. B. Kempenaers *et al.*, *Nature* **357**, 494 (1992); K. Norris, *ibid.* **362,** 537 (1993); A. P. Møller, *Proc. Natl. Acad. Sci. U.S.A.* **91**, 6929 (1994); D. Hasselquist, S. Bensch, T. von Schantz, *Nature* **381**, 229 (1996).
4. M. Petrie, *Nature* **371**, 598 (1994); B. C. Sheldon, J. Merilä, A. Qvarnström, L. Gustafsson, H. Ellegren, *Proc. R. Soc. London Ser. B Biol. Sci.* **264,** 297 (1997).
5. N. Burley, *Am. Nat.* **132**, 611 (1988); F. De Lope and A. P. Møller, *Evolution* **47**, 1152 (1993).
6. H. Schwabl, *Proc. Natl. Acad. Sci. U.S.A.* **90**, 11446 (1993).
7. _____, *J. Exp. Zool.* **276**, 157 (1996).
8. _____, D. A. Mock, J. A. Gieg, *Nature* **386,** 231 (1997).
9. J. L. Lipar, E. D. Ketterson, V. Nolan, *Auk* **116**, 231 (1999).
10. H. Schwabl, *Comp. Physiol. Biochem.* **114**, 271 (1996).
11. N. Burley, *Evolution* **40**, 1191 (1986); I. C. Cuthill *et al.*, *Proc. R. Soc. London Ser. B Biol. Sci.* **264**, 1093 (1997).
12. On the morning that each egg was laid, it was removed and replaced with a dummy egg. Clutches were considered complete once laying had stopped for 3 days or six eggs had been laid (*20*). Each pair of birds was housed in individual cages, without visual contact with other birds. The room was lit with full-spectrum fluorescent tubes. The failure of some studies to replicate an effect of band color on male attractiveness is most likely due to inadequate housing conditions without ultraviolet (UV) light (*21*). Our experiment was done under the required conditions of UV light.
13. The whole yolk was removed, homogenized in 0.5 ml of distilled water, and frozen on the morning of laying. Free steroids were extracted twice from yolk-water homogenate with 3 ml of ether. Ether fractions were decanted from the snap-frozen egg yolk–water phase, pooled, and then dried under a stream of nitrogen. The dried extract was redissolved in 90% ethanol and kept overnight at −20°C. Precipitated proteins and lipids were separated by decanting after 5 min of centrifugation at 1300*g*, and the ethanol was dried under a stream of nitrogen. The dried extract was redissolved in 1.5 ml of assay buffer. Extraction efficiencies were assessed in each assay and were on average 70%. Testosterone (T) + 5α-dihydrotestosterone (DHT) concentrations were measured by radioimmunnoassay. The established intraassay coefficient of variation is 5.0%, and the interassay coefficient of variation is 11.3% [BIOTRAK testosterone/dihydrotestosterone (3H) assay system Code TRK 600; Amersham Pharmacia Biotech]. The measured intraassay coefficient of variation was 3.9% for T + DHT and 2.0% for DHT alone. Disintegrations per minute was used throughout.
14. R. E. Hegner and J. C. Wingfield, *Horm. Behav.* **20**, 313 (1986).
15. N. L. Staub and M. De Beer, *Gen. Comp. Endocrinol.* **108**, 1 (1997); W. A. Searcy, *Ecology* **69**, 85 (1988).
16. I. Folstad and A. J. Karter, *Am. Nat.* **139**, 603 (1992); N. Saino, A. P. Møller, A. M. Bolzern, *Behav. Ecol.* **6**, 397 (1995).
17. W. C. Gause and J. A. Marsh, *Clin. Immunol. Immunopathol.* **39**, 464 (1986); A. H. W. M. Schuurs, H. Dietrich, J. Gruber, G. Wick, *Int. Allergy Immunol.* **97**, 337 (1992).
18. Reviewed by N. Hillgarth and J. C. Wingfield, in *Host-Parasite Evolution*, D. H. Clayton and J. Moore, Eds. (Oxford Univ. Press, Oxford, 1997), pp. 78–104; A. I. Al-Afaleq and A. M. Homeida, *Immunopharm. Immunotoxicol.* **20**, 315 (1998); S. Verhulst, S. J. Dieleman, H. K. Parmentier, *Proc. Natl. Acad. Sci. U.S.A.* **96**, 4478 (1999); A. F. H. Ros, T. G. G. Groothuis, V. Apanius, *Am. Nat.* **150**, 201 (1997); D. Hasselquist, J. A. Marsh, P. W. Sherman, J. C. Wingfield, *Behav. Ecol. Sociobiol.* **45**, 167 (1999).
19. D. W. Mock and G. A. Parker, *The Evolution of Sibling Rivalry* (Oxford Univ. Press, Oxford, 1997).
20. R. A. Zann, *The Zebra Finch* (Oxford Univ. Press, Oxford, 1966).
21. A. T. D. Bennett *et al.*, *Nature* **380**, 433 (1996); S. Hunt *et al.*, *Anim. Behav.* **54**, 1383 (1997).

22. We thank S. Buckland, M. Ritchie, and P. Gardner for statistical advice and comments on the manuscript. D.G. was supported by a grant to J.G. from the School of Environmental and Evolutionary Biology, University of St. Andrews, UK.

14 April 1999; accepted 24 August 1999

Neanderthal Cannibalism at Moula-Guercy, Ardèche, France

Alban Defleur,[1,2]* Tim White,[2] Patricia Valensi,[3] Ludovic Slimak,[4] Évelyne Crégut-Bonnoure[5]

The cave site of Moula-Guercy, 80 meters above the modern Rhone River, was occupied by Neanderthals approximately 100,000 years ago. Excavations since 1991 have yielded rich paleontological, paleobotanical, and archaeological assemblages, including parts of six Neanderthals. The Neanderthals are contemporary with stone tools and faunal remains in the same tightly controlled stratigraphic and spatial contexts. The inference of Neanderthal cannibalism at Moula-Guercy is based on comparative analysis of hominid and ungulate bone spatial distributions, modifications by stone tools, and skeletal part representations.

Baume ("cave") Moula-Guercy is in southeastern France on the west bank of the Rhone River, in Ardèche. The stratigraphic sequence is exclusively Middle Paleolithic. A test excavation in 1991 revealed 12 hominid skeletal fragments, some with cut marks (*1*).

The lowest exposed units (levels XVI to XX) represent a cold period that is biochronologically dated to the terminal Middle Pleistocene (isotope stage 6). The upper units (levels IV to XI) represent a cool period corresponding to isotope stage 4. Level VI is volcanic tephra dated to 72,000 ± 12,000 years ago (*2*). A thick and homogenous deposit (levels XII through XV) between the upper and lower units contains an abundant fauna representative of a temperate forest. We interpret the data to indicate an Eemian age for the latter deposits (isotope stage 5, 80,000 to 120,000 years ago) (*2*). The Neanderthal fossils all derive from level XV, a temporary Mousterian (Middle Paleolithic) occupation thought to date to between 100,000 and 120,000 years ago on the basis of biochronologies of large and small mammals (*2*). Approximately 30% of the estimated volume of this unit has been excavated (Fig. 1). Contemporary European sites are rare. Moula-Guercy's detailed paleoenvironmental and behavioral records complement its Neanderthal remains in illuminating the transition from the Middle to the Upper Pleistocene.

Level XV contains a lithic assemblage attributable to the Ferrassie Mousterian, a lithic tradition based on a high frequency of

[1]UMR 6569 du CNRS, Laboratoire d'Anthropologie, Faculté de Médecine, Secteur Nord, Boulevard Pierre Dramart, 13916 Marseille Cedex 20, France. [2]Laboratory for Human Evolutionary Studies, Museum of Vertebrate Zoology and Department of Integrative Biology, University of California, Berkeley, CA 94720, USA. [3]Laboratoire de Préhistoire du Lazaret, 33 bis Boulevard Franck Pilatte, 06300 Nice, France. [4]UMR 6636 du CNRS, 5 Rue du Chateau de l'Horloge, Boite Postale 647, 13094 Aix-en-Provence Cedex 2, Université de Provence, Aix-Marseille I, France. [5]Museé Requien, Rue Joseph Vernet, 84000, Avignon, France.

*To whom correspondence should be addressed. E-mail: defleur@voltaire.timone.univ-mrs.fr