Science
AAAS

The Domestication Process and Domestication Rate in Rice: Spikelet Bases from the Lower Yangtze
Dorian Q Fuller, *et al.*
Science **323**, 1607 (2009);
DOI: 10.1126/science.1166605

brood relative to genetically incompatible mis-matched (black-red) pairs (clutch: $t = 5.6$; egg size: $t = 6.8$; care: $t = 8.2$; df = 86, $P < 0.001$) and instead invested similarly to genetically compatible black pairs (clutch: $t = 1.2$; egg size: $t = 0.8$; care: $t = 0.5$; df = 94, $P > 0.15$). Similarly, red females paired to blackened red males (i.e., genetically compatible) significantly reduced their investment compared with matched red morph pairs (clutch: $t = 4.4$; egg size: $t = 5.7$; care: $t = 7.3$; df = 56, $P < 0.001$), in line with red females paired to genetically incompatible black males (clutch: $t = 0.9$; egg size: $t = 1.1$; care: $t = 0.7$; df = 102, $P > 0.18$).

By controlling for individual effects (i.e., within-female) and environmental effects (i.e., controlled cage environment), we demonstrated differences in maternal investment (offspring number, quality, and sex) in a system where the fitness benefits to females from skewed allocation are large (due to genetic incompatibility) and predictable (because head color is a reliable signal of genotype). By manipulating parameters pertinent to strategic maternal investment and by preventing active precopulatory mate choice (16) or the ability for females to engage in extra-pair copulations with a more compatible mate to counteract the costs of a genetically incompatible social mate (21), we exposed extreme postcopulatory strategies. This suggests that the extent to which females can control and manipulate important life-history components of fitness may have previously been underestimated.

References and Notes

1. R. L. Trivers, D. E. Willard, *Science* **179**, 90 (1973).
2. G. C. Williams, *Am. Nat.* **100**, 687 (1966).
3. N. Burley, *Evolution* **40**, 1191 (1986).
4. B. C. Sheldon, S. Andersson, S. C. Griffith, J. Örnborg, J. Sendecka, *Nature* **402**, 874 (1999).
5. J. G. Ewen, P. Cassey, A. P. Møller, *Proc. R. Soc. London Ser. B* **271**, 1277 (2004).
6. T. W. Fawcett, B. Kuijper, I. Pen, F. J. Weissing, *Behav. Ecol.* **18**, 71 (2007).
7. J. Komdeur, I. Pen, *Philos. Trans. R. Soc. London Ser. B* **357**, 373 (2002).
8. E. Cunningham, A. Russell, *Nature* **404**, 74 (2000).
9. F. de Lope, A. P. Møller, *Evolution* **47**, 1152 (1993).
10. D. Gil, J. Graves, N. Hazon, A. Wells, *Science* **286**, 126 (1999).
11. M. Petrie, A. Williams, *Proc. R. Soc. London Ser. B* **251**, 127 (1993).
12. B. C. Sheldon, *Trends Ecol. Evol.* **15**, 397 (2000).
13. S. A. West, B. C. Sheldon, *Science* **295**, 1685 (2002).
14. S. R. Pryke, S. C. Griffith, *Evolution* **63**, 793 (2009).
15. H. N. Southern, *J. Genet.* **47**, 51 (1945).
16. S. R. Pryke, S. C. Griffith, *J. Evol. Biol.* **20**, 1512 (2007).
17. Materials and methods are available as supporting material on *Science* Online.
18. S. C. Griffith, I. P. F. Owens, T. Burke, *Nature* **400**, 358 (1999).
19. T. R. Birkhead, A. P. Møller, *Biol. J. Linn. Soc. London* **50**, 295 (1993).
20. E. L. Charnov, *The Theory of Sex Allocation* (Princeton Univ. Press, Princeton, NJ, 1982).
21. T. Veen *et al.*, *Nature* **411**, 45 (2001).
22. W. R. Rice, *Science* **256**, 1436 (1992).
23. P. J. Cordero, S. C. Griffith, J. M. Aparicio, D. T. Parkin, *Behav. Ecol. Sociobiol.* **48**, 353 (2000).
24. T. D. Williams, *Biol. Rev. Camb. Philos. Soc.* **69**, 35 (1994).
25. We thank A. Badyaev, R. Montgomerie, T. Price, A. Russell, and B. Sinervo for comments; D. Briscoe, R. Brooks, R. Bondurianksky, T. Burke, and M. Olsson for discussion; and R. Merrill, J. Brazill-Boast, and L. A. Rollins for technical help. Funded by the Australian Research Council (grants awarded to S.R.P. and S.C.G.), a New South Global Postdoctoral Fellowship (to S.R.P.), a L'Oréal For Women in Science Fellowship (to S.R.P.), and the Save The Gouldian Fund. The Animal Care and Ethics Committee of the University of New South Wales and Macquarie University approved this research.

The Domestication Process and Domestication Rate in Rice: Spikelet Bases from the Lower Yangtze

Dorian Q Fuller,[1]* Ling Qin,[2] Yunfei Zheng,[3] Zhijun Zhao,[4] Xugao Chen,[3] Leo Aoi Hosoya,[5] Guo-Ping Sun[3]

The process of rice domestication occurred in the Lower Yangtze region of Zhejiang, China, between 6900 and 6600 years ago. Archaeobotanical evidence from the site of Tianluoshan shows that the proportion of nonshattering domesticated rice (*Oryza sativa*) spikelet bases increased over this period from 27% to 39%. Over the same period, rice remains increased from 8% to 24% of all plant remains, which suggests an increased consumption relative to wild gathered foods. In addition, an assemblage of annual grasses, sedges, and other herbaceous plants indicates the presence of arable weeds, typical of cultivated rice, that also increased over this period.

The domestication of staple cereal crops represents the major economic and ecological transition that human societies made during the Holocene (1). A key change in domestication of cereals, resulting from cultivation, was the loss of natural seed dispersal, which led to domesticated cereals with dependence on humans (2, 3). Direct evidence for the evolution of this trait in wheat and barley in Southwest Asia suggests that this process was slower than earlier hypothesized (3–5). Rice has been less well documented, but archaeological finds of rice grains and phytoliths indicate that it was an early crop in the Lower and Middle Yangtze region of China (6, 7).

Tianluoshan is a Neolithic site of the local Hemudu Neolithic culture in Zhejiang Province, China (Fig. 1). Tianluoshan is 2 to 3 m above present-day sea level, with a high belowground water table that has preserved water-logged botanical remains in some contexts, along with charred remains throughout the site. Excavations between 2004 and 2007 revealed preserved wooden posts, boat paddles, wooden and bone tools, characteristic pottery and ground-stone axes, and animal and fish remains, as well as well-preserved plant remains (8). In total, 23,615 plant remains were identified from 24 systematically sieved samples, in addition to more than 12,000 hand-picked remains. More than 50 species were identified, mainly acorns (includ-ing *Lithocarpus* and *Cyclobalanopsis* types), *Trapa* water chestnuts, foxnuts (*Euryale ferox*), and rice. Probable storage pits retained acorns (*Quercus sensu lato* and *Lithocarpus*), water chestnuts (*Trapa natans sensu lato*), foxnuts, and several other edible fruit remains and seeds. One area of excavation (K3) had preserved distinct lenses of rice husks, acorn shells, *Trapa* shells, and persimmon seeds (*Diospyros* sp.).

Large quantities of rice spikelet bases, as well as a range of small seeds of wild species that may plausibly represent the arable weeds of rice cultivation, were recovered during the systematic sorting of sediment samples. Rice increased as a percentage of the total remains from sieved samples from 8% to 18% to 24% (Fig. 2). These phases were dated by direct accelerator mass spectrometry radiocarbon dates on nuts and rice grains (fig. S1) indicating a sequence for the plant samples between ~6900 and 6600 years ago, and divided into three periods (K3 midden, layers 8 and 7, and layers 6 and 5). These data suggest that rice increased in dietary importance through time. The increase in the proportion of rice supports the hypothesis that people became increasingly reliant on rice cultivation and gradually abandoned wild resources, such as acorns and *Trapa* water chestnuts.

Distinctions between wild and domestic rice are made through observations of the spikelet bases, which show key morphological differences (9–12), although in archaeological specimens this distinction can be complicated if immature specimens were harvested. We classified spikelet bases on the basis of a comparative study of spikelet bases in 140 modern populations (13). In domesticated rice, panicles are nonshattering, which allows most grains on the plant to reach maturity before being

[1]Institute of Archaeology, University College London, London WC1H 0PY, UK. [2]School of Archaeology and Museology, Peking University, Beijing 100871, China. [3]Zhejiang Provincial Institute of Archaeology and Cultural Relics, Hangzhou 310014, China. [4]Institute of Archaeology, Chinese Academy of Social Sciences, Beijing 100710, China. [5]Research Institute for Humanity and Nature, Kyoto 603-8047, Japan.

*To whom correspondence should be addressed. E-mail: d.fuller@ucl.ac.uk

Fig. 1. Map of representative early rice finds in China, with arrow indicating Tianluoshan; the inset shows the local region of Tianluoshan and Liangzhu.
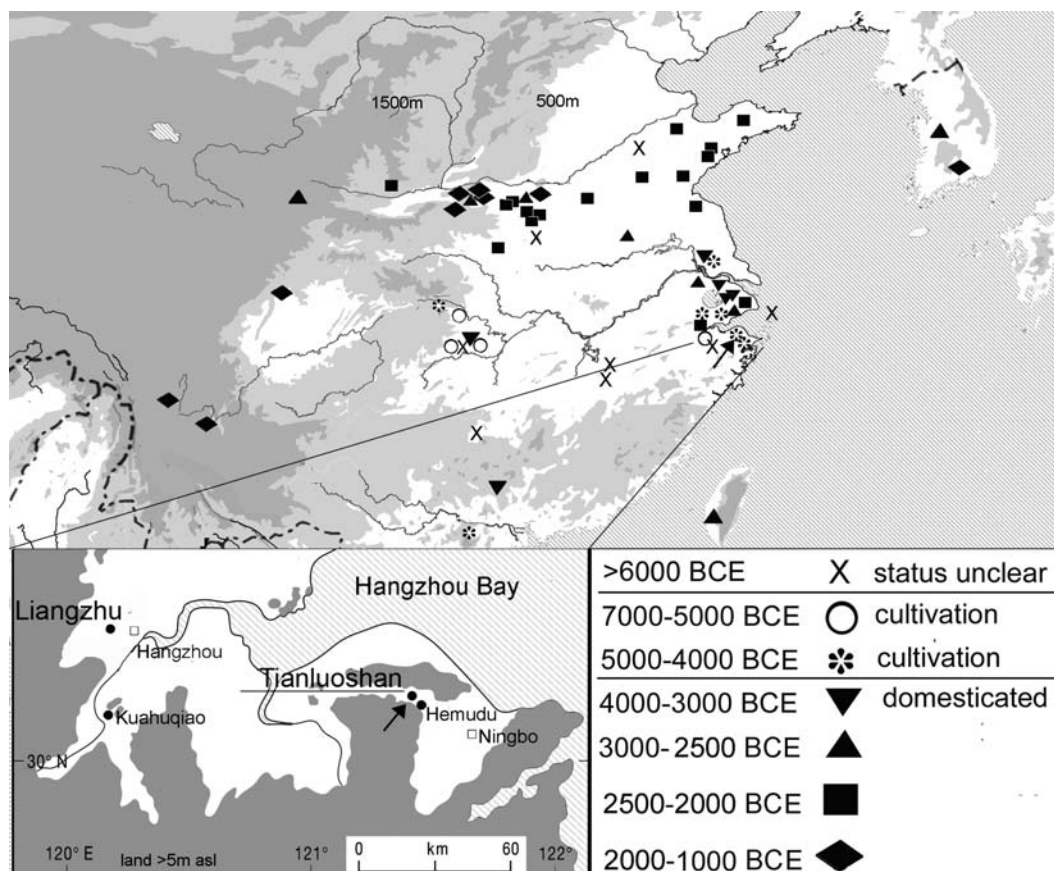
harvested. Spikelets are then separated through threshing, which causes uneven breakage at the spikelet base as well as tearing of vascular strands, resulting in a larger and more irregular pore (Fig. 3A). In addition, domesticated spikelet bases can be identified by their uneven profile, dimpled appearance, and less symmetrical scars (10). By contrast, wild-type rice spikelets typically have a straight profile at their bases, and shattering results in a smooth and round abscission scar and a small, distinct vascular pore (Fig. 3B). Rice harvested before maturity is expected to have protruding vascular bundles from the remnant of the attached rachilla (the fine stalk that attaches grains to the rice plant) (Fig. 3C), although this pattern is encountered in some modern domesticated varieties. To minimize the possibility of overestimating the proportion of domesticates, we classified seeds with rachilla remnants as immature.

On the basis of the above criteria, 2641 archaeological spikelet bases from Tianluoshan were divided into three categories: wild (Fig. 3, E and H), domestic (Fig. 3, D and G), and immature (Fig. 3, F and I); all three were found in all samples. When calculated by temporal period, the proportions change over time in favor of domesticated types, which increase from 27.4% to 38.8% over ~300 years, while both wild and immature types decrease (fig. S2). To test for statistical significance, we treated each sampled context with 25 or more spikelet bases as an independent sample, allowing a mean and standard deviation to be calculated for the percentages of domesticated, wild, and immature types

(Fig. 4). These findings were supported by a comparison with a later domesticated population: a single sample ($n = 147$) available from nearby Liangzhu [~4200 years before the present (B.P.)], a quasi-urban center of a culture known for stone plough tips and sickles (14). Our observed domesticated types may be an underestimate, because some immature types may be domesticated (13). But any such underestimate is likely to be slight because wild harvests should be biased toward immature types, as inferred from grain morphometrics (14, 15).

Through the three temporal phases at Tianluoshan, there is a significant increase in average proportion of domesticated types ($P = 0.0048$). This trend toward an increasing proportion of domesticated types through time implies that rice was under cultivation at this time and that domestication traits were under selection. However, as predicted from other lines of evidence from the region (14, 15), a substantial proportion of the rice crop may have been harvested while still immature to minimize wild-type grain loss due to shattering. We also observed many small and flattened rice grains, characteristic of highly immature spikelets, present among larger, mature grain types at Tianluoshan.

Additional support for rice cultivation at Tianluoshan is provided by the accompanying species, which include many likely arable weeds. Temporal increases in domesticated rice spikelet bases were accompanied by increases in both the overall proportion of rice and these weedy taxa (Fig. 2). These include well-known wet-field rice weeds
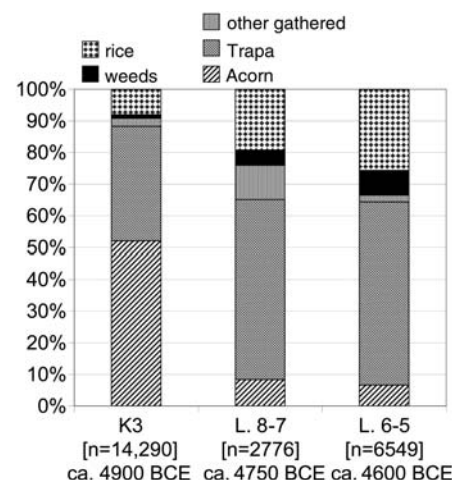
Fig. 2. Proportion of plant remains from sieved samples from the three periods, indicating percentages of rice, probable weeds of rice, acorns, *Trapa* water chestnuts, and other gathered fruits and nuts.

such as sedges (*Scirpus* spp., *Cyperus* spp., *Juncellus* spp., *Eleocharis* sp.), rushes (*Juncus* spp.), and weedy annual grasses (*Echinochloa* sp., *Eragrostis* sp., *Isachne globosa*, *Festuca* sp., *Panicum* sp., *Setaria* sp.). Several dicotyledonous weeds were also found, but with less frequency. All these species are present today as weeds in rice paddy fields (16–18).

Our data suggest that rice domestication culminated after ~6500 years B.P. This is consistent with the findings of a recent reanalysis of shifts in grain and phytolith size (3, 14, 15). The beginnings of
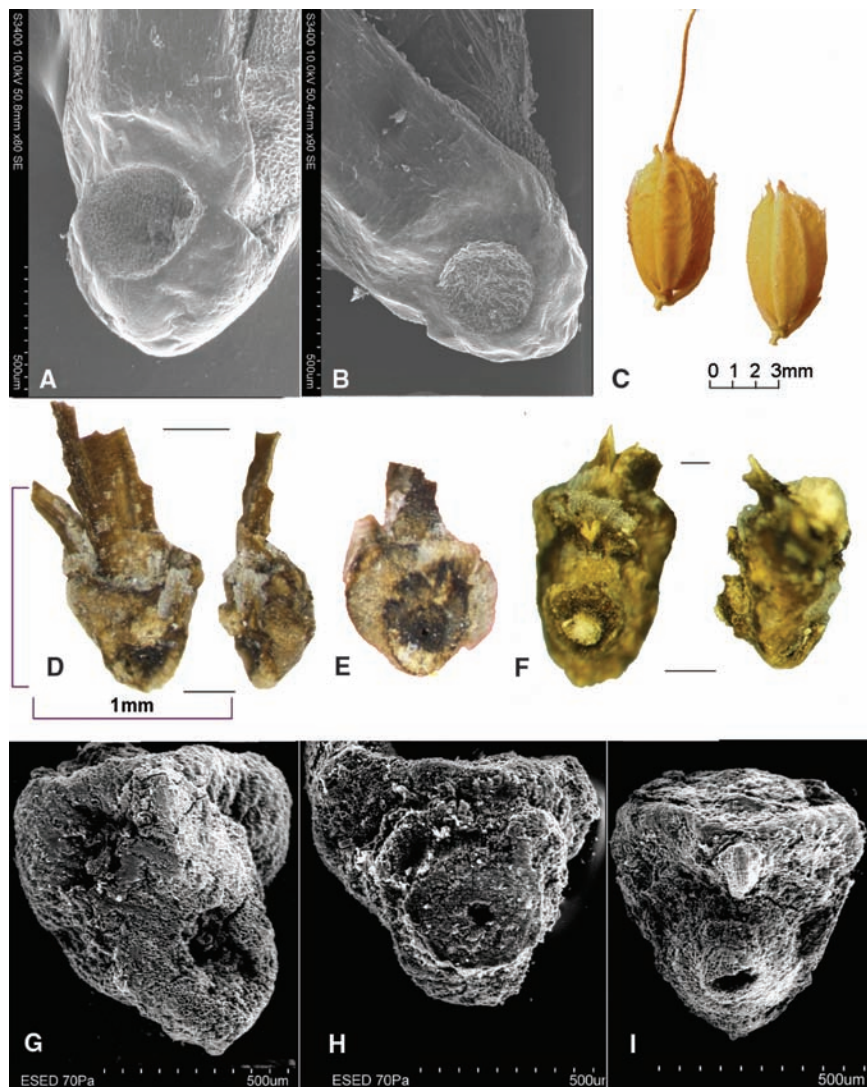
Fig. 3. Examples of modern and archaeological rice spikelet base abscission scars. (**A**) Scanning electron microscopy (SEM) image of modern *Oryza sativa* subsp. *japonica*. (**B**) SEM image of modern *Oryza rufipogon*. (**C**) Immature harvested *Oryza sativa*. (**D**) Domesticated-type spikelet base (front and profile), waterlogged, from Tianluoshan K3. (**E**) Wild type, waterlogged, from Tianluoshan K3. (**F**) Immature type (front and profile) from Tianluoshan K3. (**G**) SEM image of domesticated type, charred, from Tianluoshan H28. (**H**) SEM image of wild type, charred, from Tianluoshan H28. (**I**) SEM image of immature type, charred, from Tianluoshan H28.

Fig. 4. Proportions of wild, immature, and domesticated rice spikelet bases from three sequential periods at Tianluoshan, with later Liangzhu for comparison. Means and standard deviations are shown for the Tianluoshan periods, on the basis of all samples of 25 or more spikelet bases.
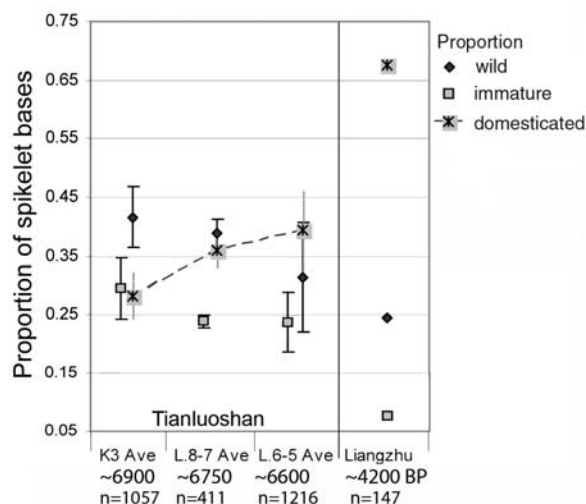
the domestication process, however, remain unclear. Early rice cultivation in China was initially a supplementary resource alongside wild nuts (*15*). Cultivation had certainly begun by 8000 to 7700 years B.P., as indicated by archaeobotanical evidence including domesticated-type spikelet bases found at Kuahuqiao (*12*). Pollen and microcharcoal data suggest that cultivation at Kuahuqiao involved water management and clearance through burning (*19*).

This evidence suggests that rice domestication was comparable in process to that of wheat and barley, in that the nonshattering phenotypes gradually became fixed in cultivated populations over at least two or three millennia (*3*, *4*). Despite higher cross-pollination rates in wild rice (*20*) relative to self-pollinating wheat and barley (*21*), pollination systems may not have had an appreciable impact on the rate of domestication. Instead, the presence of sympatric populations of both wild and domesticated cereals may have dampened selection for domestication (*22*).

Genetic studies show a deep divergence between *indica* and *japonica* rice (*23*, *24*), and it is possible that India paralleled the Chinese domestication (*25*). However, shared alleles (*26–28*) suggest that the domesticated Indian forms resulted from hybridization as domestic rice dispersed from China into South Asia. Additionally, the spread of rice to Southeast Asia derived from rice domesticated in the Yangtze (*1*). These new data from Tianluoshan would therefore restrict the time frame for dispersal until some centuries after ~6600 years B.P.

References and Notes
1. P. Bellwood, *First Farmers* (Blackwell, Oxford, 2005).
2. J. Harlan, J. De Wet, E. Price, *Evolution* **27**, 311 (1973).
3. D. Q. Fuller, *Ann. Bot.* **100**, 903 (2007).
4. K.-I. Tanno, G. Willcox, *Science* **311**, 1886 (2006).
5. E. Weiss, M. E. Kislev, A. Hartmann, *Science* **312**, 1608 (2006).
6. G. Crawford, in *Archaeology of Asia*, S. Stark, Ed. (Blackwell, Oxford, 2005), pp. 77–95.
7. T. L.-D. Lu, *Asian Perspect.* **45**, 129 (2006).
8. Zhejiang Provincial Institute of Archaeology *et al.*, *Wen Wu* **2007–11**, 4 (2007) (in Chinese).
9. G. B. Thompson, in *South-East Asian Archaeology 1992*, R. Ciarla, F. Rispoli, Eds. (Instituto Italiano per L'Africa e L'Orient, Rome, 1997), pp. 159–174.
10. C. Li, A. Zhou, T. Sang, *Science* **311**, 1936 (2006); published online 8 March 2006 (10.1126/science.1123604).
11. K. Onishi, K. Takagi, M. Kontani, T. Tanaka, Y. Sano, *Genome* **50**, 757 (2007).
12. Y. Zheng, G. Sun, X. Chen, *Chin. Sci. Bull.* **52**, 1654 (2007).
13. See supporting material on *Science* Online.
14. D. Q. Fuller, L. Qin, E. L. Harvey, in *Past Human Migrations in East Asia*, A. Sanchez-Mazas, R. Blench, M. D. Ross, I. Peiros, M. Lin, Eds. (Routledge, London, 2008), pp. 40–83.
15. D. Q. Fuller, E. L. Harvey, L. Qin, *Antiquity* **81**, 316 (2007).
16. Y.-H. Li, *Zhongguo Zacao Zhi* (Chinese Weed Flora) (Chinese Agriculture Press, Beijing, 1998) (in Chinese).
17. K. Moody, *Weeds Reported in Rice in South and Southeast Asia* (IRRI, Manila, 1991).
18. M. Galinato, K. Moody, C. Piggin, *Upland Rice Weeds of South and Southeast Asia* (IRRI, Manila, 1999).
19. Y. Zong *et al.*, *Nature* **449**, 459 (2007).
20. H.-I. Oka, H. Morishima, *Evolution* **21**, 249 (1967).
21. G. C. Hillman, S. Davies, *Biol. J. Linn. Soc.* **39**, 39 (1990).

22. R. G. Allaby, D. Q. Fuller, T. A. Brown, *Proc. Natl. Acad. Sci. U.S.A.* **105**, 13982 (2008).
23. A. J. Garris, T. Tai, J. Coburn, S. Kresovich, S. McCouch, *Genetics* **169**, 1631 (2005).
24. J. P. Londo, Y.-C. Chiang, K.-H. Hung, T.-Y. Chiang, B. Schaal, *Proc. Natl. Acad. Sci. U.S.A.* **103**, 9578 (2006).
25. D. Q. Fuller, *J. World Prehist.* **20**, 1 (2006).
26. T. Sang, S. Ge, *J. Integr. Plant Biol.* **49**, 760 (2007).
27. M. Sweeney, S. McCouch, *Ann. Bot.* **100**, 951 (2007).
28. D. A. Vaughan, B.-R. Lu, N. Tomooka, *Plant Sci.* **174**, 394 (2008).
29. Supported by grants from the British Academy, the Chinese Education Ministry, and the Zhejiang Provincial Institute of Archaeology and Cultural Relics. We thank S. Colledge, M. Wollstonecroft, and anonymous reviewers for helping to improve this text.

Variants of the Antibody Herceptin That Interact with HER2 and VEGF at the Antigen Binding Site

Jenny Bostrom,[1,2] Shang-Fan Yu,[3] David Kan,[3] Brent A. Appleton,[1] Chingwei V. Lee,[1,2] Karen Billeci,[4] Wenyan Man,[1] Franklin Peale,[5] Sarajane Ross,[3] Christian Wiesmann,[1] Germaine Fuh[1,2]*

The interface between antibody and antigen is often depicted as a lock and key, suggesting that an antibody surface can accommodate only one antigen. Here, we describe an antibody with an antigen binding site that binds two distinct proteins with high affinity. We isolated a variant of Herceptin, a therapeutic monoclonal antibody that binds the human epidermal growth factor receptor 2 (HER2), on the basis of its ability to simultaneously interact with vascular endothelial growth factor (VEGF). Crystallographic and mutagenesis studies revealed that distinct amino acids of this antibody, called bH1, engage HER2 and VEGF energetically, but there is extensive overlap between the antibody surface areas contacting the two antigens. An affinity-improved version of bH1 inhibits both HER2- and VEGF-mediated cell proliferation in vitro and tumor progression in mouse models. Such "two-in-one" antibodies challenge the monoclonal antibody paradigm of one binding site, one antigen. They could also provide new opportunities for antibody-based therapy.

The binding of antibodies to specific single antigens has prompted their use for numerous targeted therapies (*1*). However, the notion of an antibody that recognizes more than one antigen is intriguing. It has been suggested that multi-specificity may evolve and play a role in the highly efficient antibody repertoire for immune protection with one antibody performing more than one task (*2*, *3*). However, the few examples of such multi-specificity are limited to antibodies that bind small haptens (*4*), and a strategy to generate a single antigen binding fragment (Fab) capable of recognizing two unrelated proteins has not yet been reported.

We set out to explore whether dual specific antibodies can be derived from a monospecific antibody with the following approach: A repertoire of Herceptin (Genentech, South San Francisco, CA) antibody variants with mutations in the light chain (LC) complementarity determining regions (CDRs) were generated, and Fabs that can bind a new protein antigen while maintaining human epidermal growth factor receptor 2 (HER2) binding were identified. The approach is based on the understanding that modifications of the LC sequence can modulate the binding specificity of antibodies (*5*, *6*). In addition, many antibodies, with Herceptin as a prime example (*7*, *8*), bind the antigens by using mainly the heavy chain (HC) CDRs, suggesting that mutations in the LC CDRs might allow preservation of the original antigen binding specificity.

The framework regions of Herceptin variable domains (V_H and V_L, the variable domain of HC and LC, respectively) belong to subtypes that are prevalent in the human antibody repertoire (V_H3, $V_L$kappa1). Thus, mutations within the antigen binding site of Herceptin that confer a second specificity may indicate a potential for a dual specific antibody to evolve from the natural repertoire. As Herceptin is a validated therapeutic for breast cancers that overexpress HER2 (*9*), recruitment of a second binding specificity to Herceptin may add to it a distinct pharmacological activity.

[1]Department of Protein Engineering, Genentech, 1 DNA Way, South San Francisco, CA 94080, USA. [2]Department of Antibody Engineering, Genentech, 1 DNA Way, South San Francisco, CA 94080, USA. [3]Department of Translational Oncology, Genentech, 1 DNA Way, South San Francisco, CA 94080, USA. [4]Department of Assay and Automation Technology, Genentech, 1 DNA Way, South San Francisco, CA 94080, USA. [5]Department of Pathology, Genentech, 1 DNA Way, South San Francisco, CA 94080, USA.

*To whom correspondence should be addressed. E-mail: gml@gene.com

Table 1. The representative antibodies from the LC library of Herceptin. Mutations from Herceptin are shown in italics. Dashes indicate positions where no residue is present. Antigen binding affinity (K_d) was determined by surface plasmon resonance using Fab. NB indicates that no binding is detected. Single-letter abbreviations for the amino acid residues are as follows: A, Ala; C, Cys; D, Asp; E, Glu; F, Phe; G, Gly; H, His; I, Ile; K, Lys; L, Leu; M, Met; N, Asn; P, Pro; Q, Gln; R, Arg; S, Ser; T, Thr; V, Val; W, Trp; and Y, Tyr.

	CDR-L1										CDR-L2				CDR-L3						Specificity		Affinity (nM)	
	28	29	30	30a	30b	30c	30d	31	32	33	50	51	52	53	91	92	93	93a	93b	94	Antigen1	Antigen2	K_d 1	K_d 2
Herceptin	D	V	N	–	–	–	–	T	A	V	S	A	S	F	H	Y	T	–	–	T	HER2	NB	0.1	NB
3-1	*N*	V	*W*	–	–	–	–	*D*	*W*	V	*P*	A	S	*S*	*G*	*W*	*Y*	*I*	–	*A*	VEGF	NB	15	NB
bH1	D	*I*	*P*	*R*	*S*	*I*	*S*	*G*	Y	V	*W*	*G*	S	*Y*	H	Y	T	–	–	T	HER2	VEGF	26	300
bH3	D	*I*	*G*	*L*	–	–	–	*G*	*S*	V	*W*	A	S	*Y*	H	Y	T	–	–	T	HER2	VEGF	8	19,000
bH4	D	*I*	*R*	*S*	–	–	–	*G*	*S*	V	*W*	*G*	S	*Y*	H	Y	T	–	–	T	HER2	VEGF	11	3,500
4-1	D	*I*	*W*	*N*	*R*	–	–	*R*	A	*L*	*E*	*G*	S	*S*	*G*	*G*	*S*	*Y*	*S*	*S*	DR5	NB	120*	NB
4-5	*N*	V	*G*	–	–	–	–	*R*	*P*	V	*G*	*G*	S	*S*	*Y*	*G*	*S*	*F*	*G*	T	DR5	NB	150*	NB
bD1	*N*	V	*S*	–	–	–	–	*K*	*H*	V	*W*	*G*	S	*Y*	*S*	*Y*	*S*	–	–	*G*	HER2	DR5	200*	14,000*
bD2	*N*	*I*	*R*	*N*	*G*	–	–	*G*	*G*	*L*	S	A	S	F	H	Y	T	–	–	T	HER2	DR5	100*	67,000*

*IgG was used to determine the K_d.