

The scaffold protein Dvl was previously thought to act downstream of LRP6 because *dsh* overexpression activates β -catenin signaling in *Drosophila* LRP6 (*arrow*) mutants (23) and because the constitutively active Dfz2-Arrow fusion protein is inactive in *dsh* mutants (24). The explanation for this discrepancy may be that overexpressing Dsh/Dvl leads to artificial sequestration of Axin or that the protein has multiple functions in the Wnt pathway.

Taken together, the results suggest that Dvl-mediated co-aggregation triggers LRP6 phosphorylation by CK1 γ . In this model (Fig. 4D), upon Wnt signaling Dvl aggregates form at the plasma membrane, where they co-cluster LRP6 with other pathway components including Fz, Axin, and GSK3 β , in a "LRP6-signalosome." The role of Wnt would be to bridge LRP6 and Fz (25, 5), which copolymerize on a Dvl platform. Clustering of LRP6 then provides a high local receptor concentration that triggers phosphorylation by CK1 γ and Axin recruitment.

Predictions of this model are as follows: (i) artificial oligomerization of LRP6 should activate the receptor and (ii) oligomerized LRP6 should signal independent of Dvl. Indeed, forced oligomerization of LRP6 using a synthetic multimerizer is sufficient to induce Wnt signaling, and this oligomerization bypasses the need for Dvl (25). (iii) Constitutively active LRP6 should signal independently of Dvl because its self-aggregation should bypass the need for Dvl polymers. This is

also the case as shown in reporter assays with Dvl siRNA knockdown (fig. S5, B and C), which supports previous findings (26, 25). (iv) If LRP6 aggregation is a prerequisite for phosphorylation by CK1 γ rather than its consequence, LRP6 aggregates should form even when the kinase is blocked. This is the case: Nonphosphorylated LRP6 aggregates were observed in response to Wnt treatment in cells transfected with dominant-negative CK1 γ (Fig. 4C). The model of LRP6-signalosomes not only provides a mechanism for Wnt signal transduction but may also be relevant for the understanding of intracellular transport of maternal Wnt determinants in the fertilized *Xenopus* egg (27).

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28. We thank R. Pepperkok for support in the EMBL Advanced Light Microscopy Facility; the Nikon Imaging Center at the University of Heidelberg and M. Boutros and D. Ingelfinger for help with siRNA experiments; A. Glinka for advice; N. Maltry for technical help; and J. Axelrod, A. Helenius, J. Nathans, R. Nusse, T. Schwarz-Romond, and M. Semenov for reagents. This work was supported by the European Union (Endotrack) and the Deutsche Forschungsgemeinschaft.

Supporting Online Material

www.sciencemag.org/cgi/content/full/316/5831/1619/DC1
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1 November 2006; accepted 11 May 2007
10.1126/science.1137065

Neural Responses to Taxation and Voluntary Giving Reveal Motives for Charitable Donations

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Civil societies function because people pay taxes and make charitable contributions to provide public goods. One possible motive for charitable contributions, called "pure altruism," is satisfied by increases in the public good no matter the source or intent. Another possible motive, "warm glow," is only fulfilled by an individual's own voluntary donations. Consistent with pure altruism, we find that even mandatory, tax-like transfers to a charity elicit neural activity in areas linked to reward processing. Moreover, neural responses to the charity's financial gains predict voluntary giving. However, consistent with warm glow, neural activity further increases when people make transfers voluntarily. Both pure altruism and warm-glow motives appear to determine the hedonic consequences of financial transfers to the public good.

Every society needs public goods, but the mechanisms used to fund them vary. For example, taxation and government spending are lower in the United States than in most European countries, but philanthropy is higher (1). To economists, this charitable giving is a puzzle: Money is a good, so why are people willing to give

it away? One possible explanation is in terms of a "pure altruism" motive (2). Individuals with such a motive receive satisfaction from increases in a public good, such as the provision of basic services to the needy. This altruistic concern provides a motive to give, but there is also an incentive to keep money for oneself, because the cost of such charity is entirely paid by the giver, whereas the benefits are spread out over all those people who care about the needy. Only those people with a very large pure altruism motive would give voluntarily, and taxation is the normal social solution to the resulting free-riding. Pure altruism implies that people should get

some satisfaction even when public goods are supplied through mandatory taxation, because, by this account, people care only about how much of the public good is provided and not about the process by which the transfer occurs. A second possible motive for charitable giving is the sense of agency associated with the act of voluntary giving. This reward from giving has been termed "warm glow" (3, 4). If givers were driven exclusively by the warm-glow motive, they should derive satisfaction from making a voluntary gift, rather than from the increase in the level of the public good itself. On the other hand, taxation should not produce a warm glow, because paying taxes typically does not involve a voluntary choice.

The distinction between pure altruism and warm-glow motives for giving is important for several reasons. First, if giving is motivated by pure altruism, tax-funded government expenditures to provide a public good will reduce private giving, potentially dollar for dollar, as people cut their voluntary contributions in response to these higher taxes (5). There should be no similar effect with warm-glow givers, as their benefit derives from the amount of their gift. Second, a warm-glow motive for altruism provides an argument in favor of policies that encourage voluntary giving, because the warm-glow benefit provides a reward to the giver that exceeds the benefit from paying an equivalent amount in taxes (6).

Neural evidence may help clarify the relative importance of pure altruism and warm-glow motives for charitable giving. Although there is

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considerable evidence linking neural activity in the ventral striatum and the insulae to the processing of concrete rewards such as money, food, and drugs, less is known about how the brain processes more abstract rewards such as those often provided by public goods. For money, activity in the ventral striatum increases as people anticipate increases in payoffs and when they receive unexpected increases in payoffs (7, 8).

Neural responses in the ventral striatum and insulae to information about products and their prices also predict purchase decisions (9). This work supports the theory that these areas provide information on the relative rewards of different outcomes, which serve as an input to decisions about consumption and tradeoffs regarding risk and money (10). Other studies have shown that activity in the ventral striatum and the insulae is

correlated with more abstract rewards, including social rewards such as punishing unfair players in sharing games (11), voluntary contributions to charities (12, 13), and decisions to trust others (14, 15). These results motivate our focus on the ventral striatum and the insulae.

To test for the pure altruism and warm-glow motives, we used functional magnetic resonance imaging while subjects played a dictator game. Subjects received \$100 and then made decisions about whether or not to give money to a local food bank. They also observed mandatory, tax-like transfers of their money to the food bank (Fig. 1, A and B) (16). The behavioral results in this experiment are consistent with economic theory and are similar to those reported in earlier economic experiments (17–19). As shown in Fig. 2A, increases in the amounts going to the charity and decreases in the cost to the giver both increased the likelihood that a voluntary transfer was accepted. Self-reported satisfaction with the transaction followed the same pattern in both the voluntary and the mandatory conditions (Fig. 2B).

To investigate the neural activity associated with pure altruism, we used data from the mandatory treatments, which involved exogenous changes in subject and charity payoffs. Contrasts of parameter estimates (Fig. 3) show that activation in very similar areas of the ventral striatum increased with the monetary payoff to both the subject and to the charity. Regression analyses to explain activation data extracted from anatomical regions of interest (ROIs) show the same result (table S4) (16). This is the first evidence we know of demonstrating that mandatory taxation for a good cause can produce activation in specific brain areas that have been tied to concrete, individualistic rewards.

The pure altruism model predicts that people who highly value increases in the charity's payoff, relative to the value they place on getting money for themselves, will be more likely to give. The evidence economists have typically used to support this model has been indirect: Relative values have been inferred from observed decisions (20). Our experiment allowed us to observe brain activation, in areas known to respond to rewards, as we varied the money the subject received and the money the charity received. This provides a direct test of the model: Do across-subject differences in neural responses to subject and charity payoffs predict who is more likely to give to the charity?

We are able to address this question out of treatment by using neural responses in those “pure” mandatory conditions where only the subject or only the charity got money (orange and green cells, respectively, in Fig. 1B). These responses potentially serve as an indicator of how much subjects valued money for themselves and for the charity. In fact, regression coefficients show that subjects with larger activation responses to money for themselves were less likely to give to the charity (black columns in Fig. 4A), and subjects with larger activation responses to money for the charity were more likely to give (gray columns in Fig. 4A). To illustrate this rela-

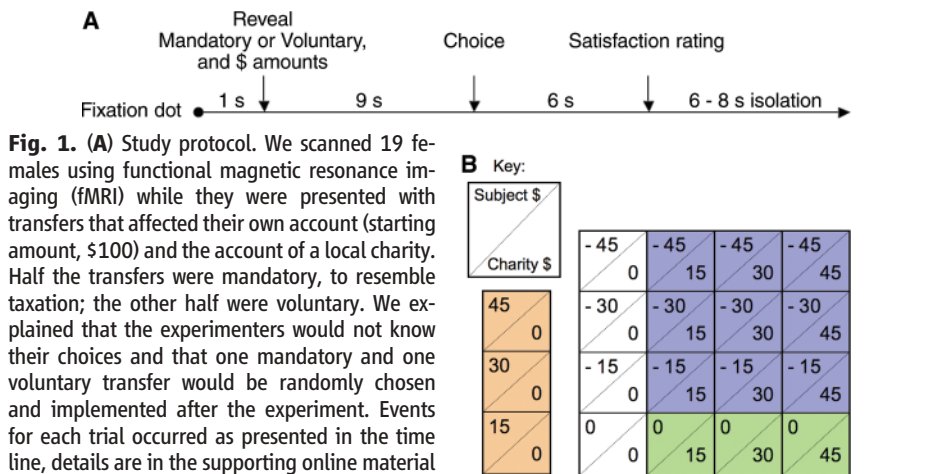


Fig. 1. (A) Study protocol. We scanned 19 females using functional magnetic resonance imaging (fMRI) while they were presented with transfers that affected their own account (starting amount, \$100) and the account of a local charity. Half the transfers were mandatory, to resemble taxation; the other half were voluntary. We explained that the experimenters would not know their choices and that one mandatory and one voluntary transfer would be randomly chosen and implemented after the experiment. Events for each trial occurred as presented in the time line, details are in the supporting online material (16). After a 1-s fixation dot, the screen revealed whether this trial's transfer was mandatory or voluntary, as well as the dollar amount change to the accounts of the subject and the charity. After 9 s, two vertically aligned labels were added in the lower portion of the screen, specifying the vertically aligned buttons on a response box. For mandatory transfers, one of the labels read “acknowledge” and the other “invalid button.” For voluntary transfers, one of the labels read “accept” and the other “reject.” Label positions varied randomly from trial to trial. Immediately after the subject's response, a four-point satisfaction rating scale was shown, to which subjects responded by pressing one of four laterally oriented keys on the button box. The rating scale disappeared after 6 s, and there was a blank screen for an intertrial period that was randomly jittered between 6, 7, and 8 s. **(B)** Study design. The cells show the dollar transfers. Each design cell was implemented three times as a mandatory transfer and three times as a voluntary decision. Orange cells indicate pure gains to the subject; green cells indicate pure gains to the charity. These pure-gain design cells from the mandatory condition were used to predict voluntary giving in the purple cells, where there was a tradeoff between the subject and the charity (see Fig. 4, A and B).

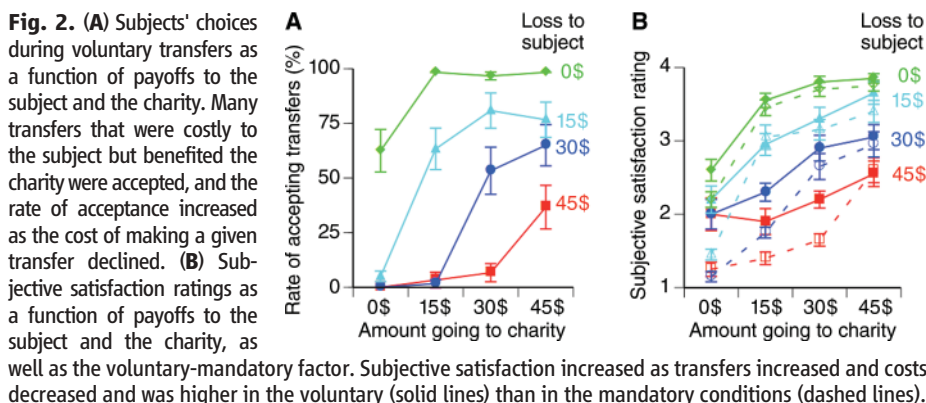


Fig. 2. (A) Subjects' choices during voluntary transfers as a function of payoffs to the subject and the charity. Many transfers that were costly to the subject but benefited the charity were accepted, and the rate of acceptance increased as the cost of making a given transfer declined. **(B)** Subjective satisfaction ratings as a function of payoffs to the subject and the charity, as well as the voluntary-mandatory factor. Subjective satisfaction increased as transfers increased and costs decreased and was higher in the voluntary (solid lines) than in the mandatory conditions (dashed lines).

Fig. 3. Neural response in the ventral striatum to mandatory payoffs for the subject (yellow), the charity (blue), and both (green).



tionship graphically, in Fig. 4B we plotted individual acceptance rates against the difference between the neural response to pure charity gains and pure subject gains, pooled over all the regions in Fig. 4A. We also split the sample into “altruists” ($n = 10$) and “egoists” ($n = 9$) depending on whether they had a larger neural response to the charity's payoff or to their own payoff. Altruists gave money nearly twice as often as egoists (58% versus 31%, $P = 0.015$). This supports the existence of a purely altruistic motive: The larger a person's neural response to increases in the public good, no matter the source, the more likely they will give voluntarily.

How then is voluntary giving different from tax-like transfers? Reported satisfaction ratings were about 10% higher for voluntary than for the mandatory transfers ($P < 0.01$, see Fig. 2B and table S2) (16). The neural evidence shows a similar result; t tests indicate higher activation in the caudate (left, $P = 0.015$; right, $P = 0.004$); the right nucleus accumbens ($P = 0.01$); and the insulae (left, $P = 0.063$; right, $P = 0.075$) in the case of voluntary transfers (table S4) (16).

Of course, these results might simply reflect the basic economic principle that adding choices cannot make the decision-maker worse off. This follows because a person who likes the payoffs in a given mandatory transfer can always obtain that same result in the corresponding voluntary condition by accepting the transfer. However, if the subject does not like the proposed transfer, only the voluntary conditions give them the option of rejecting it and keeping the money. Overall, 55% of the voluntary transfers that involved a subject's giving up money to the charity (purple cells in Fig. 1B) were rejected. This led to an increase in the expected payoff to the individual of \$13 or 33%, and a decrease in the expected payoff to the charity of \$7 or 10%, relative to the mandatory condition. So, although the opportunity for free

choice means higher activation in the caudate, the left nucleus accumbens, and the insulae, as well as higher payoffs to the individual, it reduced the level of funding for the public good.

An important question, then, is to what degree the observed higher activation comes from the ability to make a choice and to what extent it results from the differences in payoffs from that choice. We looked again at the differences in activation and satisfaction ratings between the mandatory and voluntary conditions, but this time controlling for the consequences of rejection by replacing those payoff changes with \$0. The voluntary-mandatory activation difference remained reliable for the caudate (left, $P = 0.023$; right, $P = 0.011$) and the right nucleus accumbens ($P = 0.042$), even after we controlled for payoffs (table S6) (16). Also, reported satisfaction was higher for voluntary than for mandatory transfers after controlling for payoffs ($P < 0.065$ for the complete design; $P < 0.001$ using the cells involving tradeoffs, purple in Fig. 1B). The pure altruism motive for giving, along with the story about adding choices described above, would imply that there should be no mandatory-voluntary differences after controlling for the payoff effects. Thus, our results suggest that both the increased payoffs and the ability to choose lead to increased neural activity and satisfaction.

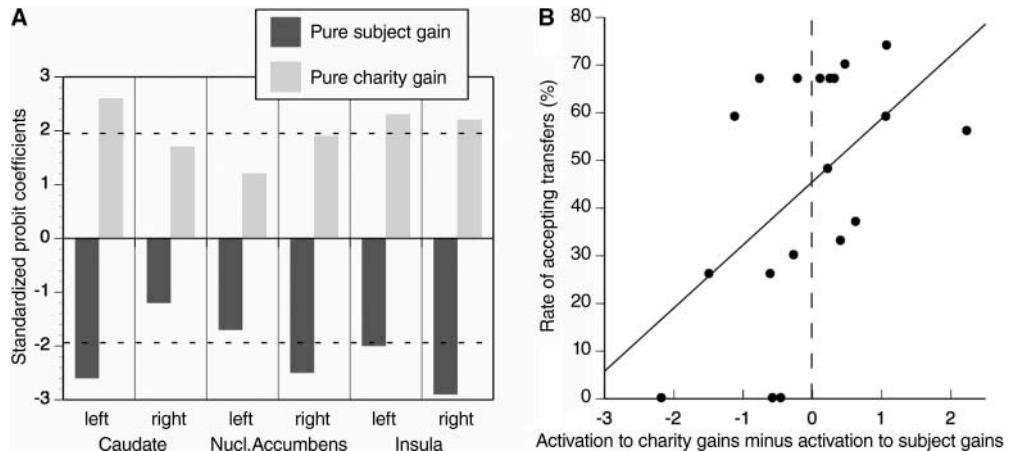
Previous results have demonstrated that activity in the areas we examined is larger when reward can be linked to one's own actions rather than to extraneous factors (21–24). Our results extend these findings about the role of agency in reward-processing to the important situation involving a choice between the subject's private payoff and the public good. What is not clear from earlier reports is whether agency-linked modulation of reward activity is actually associated with a modulation of hedonic value. Our study shows that neural activity in the caudate and right nucleus accumbens, as well as subjec-

tive satisfaction, is larger in the voluntary than in the mandatory situation. The fact that this effect persists even after controlling for payoffs supports the warm-glow theory of giving (3).

In summary, we find that three very different things—monetary payoffs to oneself, observing a charity get money, and a warm-glow effect related to free choice—all activate similar neural substrates. This result supports arguments for a common “neural currency” of reward (25–29) and shows that this model can be applied not just to choice over money, risk, and private consumption goods, but also to more abstract policy choices involving taxation and charitable giving (12). Our results are also important for understanding why people give money to charitable organizations. First, these transfers are associated with neural activation similar to that which comes from receiving money for oneself. The fact that mandatory transfers to a charity elicit activity in reward-related areas suggests that even mandatory taxation can produce satisfaction for taxpayers. A better understanding of the conditions under which taxation elicits “neural rewards” could prove useful for evaluating the desirability of different tax policies. Second, we show that the opportunity for free choice is associated with increased activity in regions implicated in processing rewards, as well as with higher reported satisfaction. Furthermore, this effect is not entirely accounted for by increased payoffs. In the context of charitable giving, this choice-related benefit is consistent with a warm-glow motive for giving.

In combination, these results suggest that both pure altruism and warm glow are important motives for charitable giving. Future work may reveal whether the free-choice effect found here extends to other situations, and under which conditions taxation elicits “neural” rewards. A related question is whether people who vote for a tax to provide a public good get a warm-glow benefit.

Fig. 4. (A) Predicting giving from activations in mandatory “pure-gain” conditions. We created measures of neural activation in response to “pure subject gain” and “pure charity gain” by averaging activation from the mandatory conditions where the subject received money at no cost to the charity and where the charity received money at no cost to the subject (orange and green cells, respectively, in Fig. 1B). We used these two sets of activations as independent predictors of the average acceptance rate in the nine design cells involving a tradeoff (purple cells in Fig. 1B). The figure shows standardized probit regression coefficients from models including subject and charity stakes as control variables and neural response to pure subject gains and pure charity gains as independent predictors. The dashed lines indicate $P = 0.05$ significance. Higher response to pure subject gain was consistently associated with less giving. Higher response to pure charity gains was consistently associated with more giving. Coefficients for individual predictors were reliable in seven out of 12 cases. **(B)** Differences in activation predict giving. As an overall measure, we



averaged the neural activation measures across all six brain areas and computed the difference between neural responses to the charity's pure gains and the neural responses to the subject's pure gains and green cells in Fig. 2B). Giving increased as the neural response to pure charity gains outweighed the neural response to pure subject gains ($R^2 = 27\%$, $P = 0.02$).

Last, public goods by their very nature are seldom traded in markets, and so we cannot observe the prices people will pay and then use these to measure value. The finding that neural activity predicts voluntary donations suggests that such activity could eventually help measure values and determine optimal levels of public goods.

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- Author contributions: Lead authorship was determined by a coin flip between the first two authors. Supported by the National Institute of Aging R01 AG1929601A1 and NSF SES-0112157. We would like to thank J. Andreoni, R. Bryck, T. Cameron, J. Chalmers, C. Rode, M. Taylor, and S. Frey, as well as the staff at the Lewis Center for Neuroimaging at the University of Oregon.

Supporting Online Material

www.sciencemag.org/cgi/content/full/316/5831/1622/DC1

Materials and Methods

Fig. S1

Tables S1 to S7

References

2 February 2007; accepted 9 May 2007

10.1126/science.1140738

Sequence Finishing and Mapping of *Drosophila melanogaster* Heterochromatin

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Genome sequences for most metazoans and plants are incomplete because of the presence of repeated DNA in the heterochromatin. The heterochromatic regions of *Drosophila melanogaster* contain 20 million bases (Mb) of sequence amenable to mapping, sequence assembly, and finishing. We describe the generation of 15 Mb of finished or improved heterochromatic sequence with the use of available clone resources and assembly methods. We also constructed a bacterial artificial chromosome–based physical map that spans 13 Mb of the pericentromeric heterochromatin and a cytogenetic map that positions 11 Mb in specific chromosomal locations. We have approached a complete assembly and mapping of the nonsatellite component of *Drosophila* heterochromatin. The strategy we describe is also applicable to generating substantially more information about heterochromatin in other species, including humans.

Heterochromatin is a major component of metazoan and plant genomes (e.g., ~20% of the human genome) that regulates chromosome segregation, nuclear organization, and gene expression (1–4). A thorough description of the sequence and organization of heterochromatin is necessary for understanding the essential functions encoded within this region of the genome. However, difficulties in cloning, mapping, and assembling regions rich in repetitive elements have hindered the genomic analysis

of heterochromatin (5–7). The fruit fly *Drosophila melanogaster* is a model for heterochromatin studies. About one-third of the genome is considered heterochromatic and is concentrated in the pericentromeric and telomeric regions of the chromosomes (X, 2, 3, 4, and Y) (5, 8). The heterochromatin contains tandemly repeated simple sequences (including satellite DNAs) (9), middle repetitive elements [such as transposable elements (TEs) and ribosomal DNA], and some single-copy DNA (10).

The whole-genome shotgun sequence (WGS3) was the foundation for finishing and mapping heterochromatic sequences and for elucidating the organization and composition of the nonsatellite DNA in *Drosophila* heterochromatin (5, 6). WGS3 is an excellent assembly of the *Drosophila* euchromatic sequence, but it has lower contiguity and quality in the repeat-rich heterochromatin. We undertook a retrospective analysis of these WGS3 scaffolds (11). Moderately repetitive sequences, such as transposable elements,

are well represented in WGS clones and sequence reads, but they tend to be assembled into shorter scaffolds with many gaps and low-quality regions because of the difficulty of accurately assigning data to a specific copy of a repeat. The typical WGS heterochromatic scaffold is smaller [for scaffolds mapped to an arm, N50 ranged from 4 to 35 kb (11)] than a typical WGS euchromatic scaffold (N50 = 13.9 Mb) (5). Relative to the euchromatic scaffolds, the WGS3 heterochromatic scaffolds have 5.8 times as many sequence gaps per Mb, as well as lower sequence quality.

To produce the Release 5 sequence, we identified a set of 10-kb genomic clones from a library representing 15× clone coverage by paired end reads (mate pairs) and used this set as templates to fill small gaps and improve low-quality regions (11). Higher-level sequence assembly into Mb-sized linked scaffolds used relationships determined from bacterial artificial chromosome (BAC)–based sequence tag site (STS) physical mapping (see below) and BAC end sequences. In addition to the WGS data, we incorporated data from 30 BACs (3.4 Mb); 15 BACs finished since Release 3) that were originally sequenced as part of the euchromatin sequencing effort (5, 10).

Sequence finishing resulted in fewer gaps, longer scaffolds, and higher-quality sequence relative to WGS3 (fig. S1). About 15 Mb of this sequence has been finished or improved, and 50% of the sequence is now in scaffolds greater than 378 kb (N50). Table 1 summarizes the Release 5 sequence statistics by chromosome arm. Improved sequence was generated for 145 WGS3 scaffolds, and a set of 90 new scaffolds were produced by joining or filling 694 gaps of previously unknown size between WGS3 scaffolds. The relationships between the initial WGS scaffolds and the Release 5 scaffolds can be complex (Fig. 1 and figs. S2 to S7); for example, there were eight cases in which small scaffolds were used to fill gaps within larger scaffolds, and two scaffolds whose gaps interdigitated. As expected, the sequence consists largely of nests of frag-

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