Biology Faculty Publications & Presentations   Biology

6-15-2015

Genomic Signatures of Cooperation and Conflict in the Social Amoeba

Elizabeth A. Ostrowski

Yufeng Shen

Xiangjun Tian

Richard Sucgang

Huaiyang Jiang

See next page for additional authors

Recommended Citation

Ostrowski, Elizabeth A.; Shen, Yufeng; Tian, Xiangjun; Sucgang, Richard; Jiang, Huaiyang; Qu, Jiaxin; Katoh-Kurasawa, Mariko; Brock, Debra A.; Dinh, Christopher; Lara-Garduno, Fremiet; Lee, Sandra L.; Kovar, Christie L.; Dinh, Huyen H.; Korchina, Viktoriya; Jackson, LaRonda; Patil, Shobha; Han, Yi; Chaboub, Lesley; Shaulsky, Gad; Muzny, Donna M.; Worley, Kim C.; Gibbs, Richard A.; Richards, Stephen; Kuspa, Adam; Strassmann, Joan E.; and Queller, David C., "Genomic Signatures of Cooperation and Conflict in the Social Amoeba" (2015). *Biology Faculty Publications & Presentations*. 68.
https://openscholarship.wustl.edu/bio_facpubs/68

Authors

Elizabeth A. Ostrowski, Yufeng Shen, Xiangjun Tian, Richard Sucgang, Huaiyang Jiang, Jiaxin Qu, Mariko Katoh-Kurasawa, Debra A. Brock, Christopher Dinh, Fremiet Lara-Garduno, Sandra L. Lee, Christie L. Kovar, Huyen H. Dinh, Viktoriya Korchina, LaRonda Jackson, Shobha Patil, Yi Han, Lesley Chaboub, Gad Shaulsky, Donna M. Muzny, Kim C. Worley, Richard A. Gibbs, Stephen Richards, Adam Kuspa, Joan E. Strassmann, and David C. Queller

1   **Genomic Signatures of Cooperation and Conflict in the Social Amoeba**

2

3   Elizabeth A. Ostrowski[1*], Yufeng Shen[2], Xiangjun Tian[3], Richard Sucgang[4], Huaiyang Jiang[5],

4   Jiaxin Qu[5], Mariko Katoh-Kurasawa[6], Debra A. Brock[7], Christopher Dinh[4], Fremiet Lara-

5   Garduno[5], Sandra L. Lee[5], Christie L. Kovar[5], Huyen H. Dinh[5], Viktoriya Korchina[5], LaRonda

6   Jackson[5], Shobha Patil[5], Yi Han[5], Lesley Chaboub[5], Gad Shaulsky[6], Donna M. Muzny[5,6], Kim C.

7   Worley[5,6], Richard A. Gibbs[5,6], Stephen Richards[5], Adam Kuspa[4], Joan E. Strassmann[7], and

8   David C. Queller[7].

9

10   *Author for correspondence

11

12   [1]Department of Biology and Biochemistry, University of Houston, Houston, TX 77204, USA

13   [2]Departments of Systems Biology and Biomedical Informatics, Columbia University, New York,

14   NY 10027, USA

15   [3]Alkek Center for Metagenomics and Microbiome Research, Department of Molecular Virology

16   and Microbiology, Baylor College of Medicine, Houston, TX 77030, USA

17   [4]Verna and Marrs McLean Department of Biochemistry and Molecular Biology, Baylor College of

18   Medicine, Houston, TX 77030, USA

19   [5]Human Genome Sequencing Center, Baylor College of Medicine, Houston, TX 77030 USA

20   [6]Department of Molecular and Human Genetics, Baylor College of Medicine, Houston, TX 77030

21   USA

22   [7]Department of Biology, Washington University in St. Louis, St. Louis, MO 63130, USA

23

24   ***Contact Information:** eaostrowski@uh.edu

25

1 **Running Title**

2 Genomic Signatures of Cooperation and Conflict

3

4 **Summary**

5 Cooperative systems are susceptible to invasion by selfish individuals that profit from receiving

6 the social benefits but fail to contribute. These so-called "cheaters" can have a fitness

7 advantage in the laboratory, but it is unclear whether cheating provides an important selective

8 advantage in nature. We used a population genomic approach to examine the history of genes

9 involved in cheating behaviors in the social amoeba *Dictyostelium discoideum*, testing whether

10 these genes experience rapid evolutionary change as a result of conflict over spore-stalk fate.

11 Candidate genes and surrounding regions showed elevated polymorphism, unusual patterns of

12 linkage disequilibrium, and lower levels of population differentiation, but they did not show

13 greater between-species divergence. The signatures were most consistent with frequency-

14 dependent selection acting to maintain multiple alleles, suggesting that conflict may lead to

15 stalemate rather than an escalating arms race. Our results reveal the evolutionary dynamics of

16 cooperation and cheating and underscore how sequence-based approaches can be used to

17 elucidate the history of conflicts that are difficult to observe directly.

18

19 **Highlights**

20 • Molecular evolution analyses reveal the history of social conflict

21 • Genes that mediate social conflict show signatures of frequency-dependent selection

22 • Balanced polymorphisms suggest that cheating may be stable and endemic

23

24

25 **Results**

1   The social amoeba *Dictyostelium discoideum* is a model system for cooperation and conflict [1,

2   2]. Upon starvation, up to hundreds of thousands of amoebae converge, forming a multicellular

3   slug and eventually a fruiting body. Some cells form the stalk of the fruiting body and die, while

4   others rise to the top, form resistant spores, and disperse. Stalk formation is altruistic, because

5   death of the stalk cells enhances the survival and dispersal of the spores. However, because

6   fruiting bodies can contain multiple, genetically different clones, selection can favor cheaters –

7   individuals that avoid forming the stalk themselves, yet benefit from its production by others [2].

8

9   Consistent with the prediction of cheating, cheaters can be readily found in natural populations

10   of *D. discoideum* [1-3]. However, whether individuals that cheat are evolutionarily successful is

11   unclear, and several hypotheses have been proposed. One hypothesis is that cheating selects

12   for resistance, and resistance in turn selects for greater cheating. Social conflict could thus drive

13   an escalating arms race of adaptations and counter-adaptations, reminiscent of the arms races

14   between hosts and pathogens or predators and prey [4-7]. An alternative possibility is that

15   cheaters have a selective advantage only when rare. For example, as cheaters increase in

16   frequency in a population, they potentially displace the very victims on which they depend, or

17   face other trade-offs [8]. This negative frequency-dependence predicts that cheaters and

18   cooperators can be maintained as a balanced polymorphism, effectively leading to a stalemate

19   (Fig. 1B) [9, 10]. Finally, some have suggested that there is no selective advantage to cheating

20   [11]. Cheating might be selected against if relatedness among the strains in a fruiting body is

21   high, such that cheaters primarily cheat their own relatives [12]. In this case, cheating might

22   persist in populations as a 'cheating load', analogous to a genetic load for deleterious mutations

23   (Fig. 1C). Alternatively, cheating might also not be favored if the multicellular stage occurs only

24   rarely in nature [13], such that there is little selection for or against these phenotypes.

25

1   Crucially, these different hypotheses about the long-term success of social cheating make

2   unique, testable predictions about variation in the genes that mediate these conflicts ([14]; Table

3   1). To distinguish among these different possibilities, we took advantage of a previous screen

4   that identified over >150 loci in *D. discoideum* that impact cheating behaviors [5]. We used

5   whole genome sequencing and molecular evolution to ask whether genes that mediate cheating

6   behaviors show distinctive signatures of molecular evolution that differ from the rest of the

7   genome, distinguishing among the different hypotheses described in Table 1.

8

9   **Candidate Genes Show Elevated Polymorphism**

10   The different evolutionary scenarios for cheating alleles make unique predictions about the

11   levels of polymorphism versus divergence (Table 1). For example, an escalating arms race

12   driven by repeated selective sweeps of cheating alleles should reduce variation within species

13   while elevating the sequence divergence between species, whereas the stalemate model

14   makes the opposite prediction. To test these possibilities, we first examined levels of

15   polymorphism in regions surrounding candidate genes, comparing these values to a null

16   hypothesis based on other regions of the genome. We observed higher polymorphism in

17   candidate genes as a group compared to randomly chosen regions, which was significant for

18   both mean and median levels at 20 kb (Fig. 2). Compared to other genes, sequence variation

19   was also disproportionately non-synonymous (higher p_N/p_S; Table S1). Higher levels of

20   polymorphism might occur if genes important for cheating behaviors show limited expression

21   (e.g., if they are expressed in an infrequent portion of the life cycle [13]), but analysis of the

22   published transcriptome of the lab strain [15] indicated no difference in their timing or levels of

23   expression compared to other genes in the genome (Table S2).

24

25   **No Evidence of Elevated Sequence Divergence between Species**

The arms race hypothesis also predicts elevated rates of divergence between species, so we compared *D. discoideum* to its sister species *D. citrinum* at all identified orthologs. These analyses revealed lower rates of non-synonymous to synonymous substitution (d_N/d_S) for candidate genes compared to other genes in the genome (Table S1), which was inconsistent with the predictions of an arms race (Table 1). We reached a similar conclusion using McDonald-Kreitman tests [16], which compare p_N/p_S to d_N/d_S for each gene individually. Given very low levels of sequence polymorphism within *D. discoideum*, there was little resolution to detect significant deviations in this ratio for each locus individually. Nevertheless, two candidate genes (DDB_G0285541 and *chtC*) had strongly significant McDonald-Kreitman tests that were also in the extreme tail of the genome-wide distribution. These genes showed elevations in d_N/d_S relative to p_N/p_S, indicative of directional selection driving sequence divergence. In the remainder of candidate genes (that is, removing these two genes), the ratio was strongly significant and opposite in direction, indicating an excess of non-synonymous polymorphism to non-synonymous divergence compared to other genes (Fig. 3). Taken together, genes mediating social cheating did not show the elevated rates of amino-acid substitution predicted under an escalating arms race or relaxed selection scenario.

Additional Signatures of Selection Support Stalemates

At first glance, elevated non-synonymous polymorphism, combined with low amino acid divergence, is potentially consistent with the stalemate model of frequency-dependent selection, where novel alleles can invade and establish in populations, but ultimately fail to take over. Several additional tests support this interpretation. First, scaled to gene length, we observed significantly fewer haplotypes than expected and high levels of intragenic linkage disequilibrium (lower •; Fig. S1), indicating divergent alleles, a signature of balancing selection. In addition, two

1   metrics of balancing selection, Wall's *B* and Wall's *Q* [17], were significantly elevated in

2   sequence windows surrounding candidate loci (Table S3).

3

4   A common test for balancing selection is to examine the distribution of allele frequencies –

5   whereas positive or purifying selection produce a strongly skewed distribution, balancing

6   selection can maintain multiple alleles at intermediate frequencies. Surprisingly, given our

7   results above supporting balancing selection, candidate loci showed greater skew, indicated by

8   more negative values for two metrics of the site frequency spectrum (Table S4). Candidate

9   genes as a group also showed a significant excess of high frequency derived alleles (Fay and

10   Wu's *H*: -0.002; *P*=0.03), which can indicate incomplete selective sweeps. The significant,

11   negative Fay and Wu's *H* test for candidate genes suggests that variants rise to high frequency

12   quickly and that these genes experience stronger-than-expected selection for sequence

13   changes.

14

15   Finally, we calculated the index of population structure (F_{ST}) at each segregating site in the

16   genome, comparing SNPs in close proximity to candidate genes against the rest of the genome.

17   Unusually high F_{ST} can indicate that different alleles predominate in different geographic

18   locations (in this case, Texas and Virginia) and can be caused by geographically restricted

19   selective sweeps. On the other hand, unusually low F_{ST} values indicate alleles attain similar

20   frequencies across geographically distinct populations, with each subpopulation maintaining

21   multiple divergent alleles—a signature of negative frequency-dependent selection [18]. These

22   results revealed lower-than-expected F_{ST} at candidate compared to non-candidate loci (Fig. S2).

23   The elevation in polymorphism in these genes, combined with significant •reductions in

24   population structure, argues against divergent alleles caused by local •sweeps and suggests a

role for negative frequency-dependent selection, with selection maintaining the same variants across subpopulations. •

Evidence for Multiple Modes of Selection

By examining the molecular evolution patterns of candidate genes as a group, we could determine whether genes that mediate social conflict have general, recognizable patterns indicating unique forms of selection. Analyzing these genes as a group also provided greater sensitivity, which was helpful given the low levels of polymorphism in this species. However, these results reflect only average differences between candidate and non-candidate genes – and in fact, a diversity of dynamics is possible. Moreover, for many metrics, candidate genes might be extreme in opposite directions, such that we may fail to observe a strong signal of selection because these effects average out. We addressed this possibility in two ways. First, for each evolutionary metric, we asked whether the variance was higher for candidate genes compared to groups where genes are chosen randomly, indicating extremes in opposite directions. However, we did not observe elevated variance for any metric (Table S5). We also asked whether there was overrepresentation of candidate genes in both tails of the genome-wide distribution for each metric, but we observed no such cases (Table S6). While there was no overrepresentation in either tail of the genome-wide distribution for the McDonald-Kreitman test, two genes (DDB_G0285541 and *chtC*) showed extreme signatures of positive (directional) selection. The remaining genes showed the opposite pattern, an average excess of within-species non-synonymous polymorphism compared to non-synonymous divergence. Thus, while the average signature in conflict-related genes was elevated polymorphism and other patterns suggesting the selective maintenance of multiple alleles, other signatures were evident as well, including signatures of strong directional selection that could reflect escalating arms races at these particular loci.

1

2 **Discussion**

3 In *D. discoideum*, a model system for the study of social conflict, it has long been noted that

4 cheating behaviors are present among natural isolates [2, 3], but there is little understanding of

5 why selfish behaviors arise, whether they persist, and whether the prevalence of cheaters in

6 natural populations signifies long-term evolutionary success of this social strategy. We have

7 indications of three main signatures of balancing selection on these genes as a class compared

8 to other genes. First, they show higher levels of polymorphism, as expected when at least one

9 SNP is under balancing selection and increases diversity at linked neutral loci. However, they

10 did not show the concomitant elevation in amino acid substitution expected if polymorphism

11 simply reflected relaxed selection. Second, they showed lower F_{ST} values, which can occur if

12 selection is maintaining the same balanced polymorphisms across geographically different

13 subpopulations. Third, another indicator of the deeper coalescence times characteristic of

14 balancing selection is the significantly fewer haplotypes and higher linkage disequilibrium values,

15 including significantly elevated Wall's *B* and Wall's *Q*.

16

17 The observation of elevated levels of polymorphism surrounding genes implicated in social

18 cheating, combined with other signatures of recent selection, argue that *D. discoideum*

19 experiences ongoing selection at these loci and is consistent with frequency-dependent

20 selection allowing multiple types (or alleles) to coexist. Notably, we failed to observe the

21 molecular signatures of a simple arms race – these dynamics are expected to reduce genetic

22 diversity and drive long-term sequence divergence between populations and/or species. Rather,

23 our results are reminiscent of 'trench warfare', an alternative arms race scenario where

24 alternative alleles do not rapidly displace one another, resulting in a prolonged stalemate [19].

Negative-frequency dependence is also a hallmark of Red Queen dynamics, a form of

coevolutionary arms race where alleles continually cycle but rarely fix [20].

The finding of balancing selection is also consistent with evolutionary theory about the role of

frequency-dependence in social interactions [21]. Many social behaviors are inherently

frequency-dependent, where the fitness of a given strategy (e.g., cheat or cooperate) is

dependent on whether an individual's social partners employ the same strategy or not.

Experimental studies of bacterial mutants that exhibit cheating behaviors suggest frequency-

dependence might be common [22-26]. In *Dictyostelium*, frequency-dependent fitness was

shown for the *fbxA-* strain, a mutant that allocates fewer cells to the stalk but produces

disproportionately many spores when co-developed with another strain, which it cheats [12].

Like social conflict, conflicts between the sexes over optimal levels of mating and between

parents and offspring over optimal provisioning are also hypothesized to result in antagonistic

coevolution [20, 27-29]. While some studies have shown directional selection on genes

underlying these other forms of intraspecific conflict, many others have found signatures of

balancing selection, diversifying selection, or a combination of both [30-33]. Thus taken together,

our results not only indicate stalemate as a possible outcome of social conflict, but add to a

growing body of evidence that stalemates may be a common outcome in conflict-driven systems

more generally. While identification of these polymorphisms should open the door to

investigation into the functional consequences of this variation for cheating and resistance

behaviors, the population genomic approach used here provides insight into the long-term

consequences of social conflict and highlights the possibility of an ongoing, dynamic interaction

at these loci.

1 **Experimental Procedures**

2 **Strains.** We re-sequenced the genomes of 20 natural isolates of *D. discoideum*, primarily from

3 two locations: Houston, TX (six strains) and Mountain Lake, VA (nine strains), as well as 5

4 additional strains from different geographic locations (two sites in Texas, and one site in each of

5 Massachusetts, Kentucky, and Illinois) using 454 or Illumina. Sequencing reads were aligned to

6 the Ax4 reference genome (Assembly/GFF3 file generated June 9 2010, available at

7 dictybase.org) using MAQ for Illumina sequencing reads and ATLAS-SNP for 454 data. Detailed

8 mapping and SNP calling procedures are available in the Supplemental Information.

9

10 **Molecular Evolution Analyses**. Nucleotide diversity, Tajima's *D*, Fu and Li's *D**, Hudson's *C*

11 (recombination, or rho), haplotype diversity, Fay and Wu's *H*, and haplotype number were

12 determined for all genes in the genome and in sequence windows using "compute" (available at

13 molpopgen.org). Levels of non-synonymous (p_N) and synonymous (p_S) diversity were calculated

14 using the program "gestimator", and the McDonald-Kreitman tests were obtained using "MKtest"

15 (both available at molpopgen.org). F_{ST} was calculated for all segregating sites using scripts

16 written in Ruby and Python. Resampling analyses were performed using R. More details are

17 available in the Supplemental Information.

18

19 **Author Contributions**

20 Cell culture and DNA extraction: EAO, CD, MKK, DB; Conceived and designed the experiments:

21 EAO, JES, DCQ, SR, KCW, RAG, RS; Library preparation and sequencing: FL-G, SLL, CK, HD,

22 VK, LJ, SP, YH, LC, DMM, SR, RAG, KW; Analyzed the data: EAO, YS, XT, RS, SR; Assembly

23 and annotation of *D. citrinum*: JQ, HJ, KW; Analysis of *D. citrinum* and *D. discoideum*: EAO, XT;

24 Wrote the paper: EAO, JES, AK, DCQ.

25

1 **Acknowledgements**

2 We thank Rasmus Nielsen for discussions of the data and comments on an earlier version of

3 this manuscript.

References

1. Strassmann, J. E., and Queller, D. C. (2011). Evolution of cooperation and control of cheating in a social microbe. Proc. Natl. Acad. Sci. U.S.A. *108 Suppl 2*, 10855–10862.

2. Strassmann, J. E., Zhu, Y., and Queller, D. C. (2000). Altruism and social cheating in the social amoeba *Dictyostelium discoideum*. Nature *408*, 965–967.

3. Buttery, N. J., Rozen, D. E., Wolf, J. B., and Thompson, C. R. L. (2009). Quantification of social behavior in *D. discoideum* reveals complex fixed and facultative strategies. Curr. Biol. *19*, 1373–1377.

4. Khare, A., Santorelli, L. A., Strassmann, J. E., Queller, D. C., Kuspa, A., and Shaulsky, G. (2010). Cheater-resistance is not futile. Nature *461*, 980–982.

5. Santorelli, L. A., Thompson, C. R. L., Villegas, E., Svetz, J., Dinh, C., Parikh, A., Sucgang, R., Kuspa, A., Strassmann, J. E., Queller, D. C., et al. (2008). Facultative cheater mutants reveal the genetic complexity of cooperation in social amoebae. Nature *451*, 1107–1110.

6. Hollis, B. (2012). Rapid antagonistic coevolution between strains of the social amoeba *Dictyostelium discoideum*. Proc. Biol. Sci. *279*, 3565–3571.

7. Zhang, Q.-G., Buckling, A., Ellis, R. J., and Godfray, H. C. J. (2009). Coevolution between cooperators and cheats in a microbial system. Evolution *63*, 2248–2256.

8. Sathe, S., Khetan, N., and Nanjundiah, V. (2014). Interspecies and intraspecies interactions in social amoebae. J. Evol. Biol. *27*, 349–362.

9. Maynard Smith, J. (1982). Evolution and the Theory of Games (Cambridge: Cambridge University Press).

10. Doebeli, M., Hauert, C., and Killingback, T. (2004). The evolutionary origin of cooperators and defectors. Science *306*, 859–862.

11. Van Dyken, J. D., Linksvayer, T. A., and Wade, M. J. (2011). Kin selection–mutation balance: A model for the origin, maintenance, and consequences of social cheating. Am. Nat. *177*, 288–300.

12. Gilbert, O. M., Foster, K. R., Mehdiabadi, N. J., Strassmann, J. E., and Queller, D. C. (2007). High relatedness maintains multicellular cooperation in a social amoeba by controlling cheater mutants. Proc. Natl. Acad. Sci. U.S.A. *104*, 8913–8917.

13. Linksvayer, T. A., and Wade, M. J. (2009). Genes with social effects are expected to harbor more sequence variation within and between species. Evolution *63*, 1685–1696.

14. Van Dyken, J. D., and Wade, M. J. (2012). Detecting the molecular signature of social conflict: theory and a test with bacterial quorum sensing genes. Am. Nat. *179*, 436–450.

15. Parikh, A., Miranda, E. R., Katoh-Kurasawa, M., Fuller, D., Rot, G., Zagar, L., Curk, T., Sucgang, R., Chen, R., Zupan, B., et al. (2010). Conserved developmental

transcriptomes in evolutionarily divergent species. Genome Biol. *11*, R35.

16. McDonald, J. H., and Kreitman, M. (1991). Adaptive protein evolution at the *Adh* locus in *Drosophila*. *351*, 652–654.

17. Wall, J. D. (1999). Recombination and the power of statistical tests of neutrality. *74*, 65–79.

18. Glémin, S. (2005). Balancing selection in the wild: testing population genetics theory of self-incompatibility in the rare species *Brassica insularis*. Genetics *171*, 279–289.

19. Stahl, E. A., Dwyer, G., Mauricio, R., Kreitman, M., and Bergelson, J. (1999). Dynamics of disease resistance polymorphism at the *Rpm1* locus of *Arabidopsis*. Nature *400*, 667–671.

20. Brockhurst, M. A., Chapman, T., King, K. C., Mank, J. E., Paterson, S., and Hurst, G. D. D. (2014). Running with the Red Queen: the role of biotic conflicts in evolution. Proc. Biol. Sci. *281*, 20141382.

21. Queller, D. C. (2011). Expanded social fitness and Hamilton's rule for kin, kith, and kind. Proc. Natl. Acad. Sci. U.S.A. *108*, 10792–10799.

22. Diggle, S. P., Griffin, A. S., Campbell, G. S., and West, S. A. (2007). Cooperation and conflict in quorum-sensing bacterial populations. Nature *450*, 411–414.

23. Ross-Gillespie, A., Gardner, A., West, S. A., and Griffin, A. S. (2007). Frequency dependence and cooperation: theory and a test with bacteria. Am. Nat. *170*, 331–342.

24. Dugatkin, L. A., Perlin, M., Lucas, J. S., and Atlas, R. (2005). Group-beneficial traits, frequency-dependent selection and genotypic diversity: an antibiotic resistance paradigm. Proc. Biol. Sci. *272*, 79–83.

25. Ellis, R. J., Lilley, A. K., Lacey, S. J., Murrell, D., and Godfray, H. C. J. (2007). Frequency-dependent advantages of plasmid carriage by *Pseudomonas* in homogeneous and spatially structured environments. ISME J *1*, 92–95.

26. MacLean, R. C., and Gudelj, I. (2006). Resource competition and social conflict in experimental populations of yeast. Nature *441*, 498–501.

27. Arnqvist, G., and Rowe, L. (2005). Sexual Conflict (Princeton: Princeton University Press).

28. Rice, W. R., and Holland, B. (1997). The enemies within: intergenomic conflict, interlocus contest evolution (ICE), and the intraspecific Red Queen. Behav. Ecol. Sociobiol. *41*, 1–10.

29. Trivers, R. L. (1974). Parent-offspring conflict. Am. Zool. *14*, 249–264.

30. Hamm, D., Mautz, B. S., Wolfner, M. F., Aquadro, C. F., and Swanson, W. J. (2007). Evidence of amino acid diversity–enhancing selection within humans and among primates at the candidate sperm-receptor gene *PKDREJ*. Am. J. Hum. Gen. *81*, 44–52.

1   31.   Kelleher, E. S., Clark, N. L., and Markow, T. A. (2011). Diversity-enhancing selection acts
2   on a female reproductive protease family in four subspecies of *Drosophila mojavensis*.
3   Genetics *187*, 865–876.

4   32.   Kawabe, A., Fujimoto, R., and Charlesworth, D. (2007). High diversity due to balancing
5   selection in the promoter Region of the *Medea* Gene in *Arabidopsis lyrata*. Curr. Biol. *17*,
6   1885–1889.

7   33.   Miyake, T., Takebayashi, N., and Wolf, D. E. (2009). Possible diversifying selection in the
8   imprinted gene, *MEDEA*, in *Arabidopsis*. Mol. Biol. Evol. *26*, 843–857.

9

10

1   **Figure Legends**

2

3   **Figure 1. Example scenarios for the evolutionary dynamics of cheating behaviors.**

4   Shaded areas are proportional to the frequencies of different alleles (colors) in a population. (A)

5   Escalating arms races, where epidemics of cheating and/or resistance sweep through

6   populations, (B) Stalemates, where cheaters invade but neither fix nor become lost from the

7   population, resulting in endemic cheating, (C) Mutation-selection balance, where new mutations

8   that produce cheating behaviors are continually introduced into a population, but selection

9   removes them.

10

11   **Figure 2. Comparison of polymorphism in regions surrounding candidate loci compared**

12   **to the rest of the genome.** Dotted lines show the mean or median nucleotide diversity in

13   sequence windows of 10- or 20-kb compared to the null distribution based on 10,000 data sets

14   of the same size where sequence windows were chosen randomly. Asterisks indicate

15   statistically significant results. (A) Mean nucleotide diversity in 10-kb sequence windows, (B)

16   mean nucleotide diversity in 20-kb sequence windows, (C) median nucleotide diversity in 10-kb

17   sequence windows, and (D) median nucleotide diversity in 20-kb sequence windows.

18

19   **Figure 3. McDonald-Kreitman tests.** Histograms show the mean or median ratio of d_N/d_s to

20   p_N/p_s for 10,000 randomly generated gene sets, and the dotted line shows the observed value of

21   this ratio for candidate genes. Asterisks indicate statistically significant results. (A) Mean ratio,

22   (B) median ratio, (C and D) mean and median ratios after removing two candidate genes with

23   extreme values. See text for details.

24

25

1 **Tables**

2 **Table 1. Predicted sequence patterns for cooperation and cheating genes under different**

3 **evolutionary scenarios.**

Scenario	Description	Within-species polymorphism	Between-species divergence	Additional Signatures
Escalating Arms Race (Directional Selection)	Repeated selective sweeps of cheating alleles through populations remove variation within populations and drive rapid divergence between species	↓	↑	Elevated population structure (higher F_{ST}), excess of high frequency derived alleles (negative Fay and Wu's H)
Stalemate (Balancing Selection)	Negative frequency-dependence maintains both cheaters and cooperators within populations	↑	↓	Reduced population structure (lower F_{ST}), excess of intermediate frequency alleles (positive Tajima's D), elevated linkage disequilibrium (lower •), haplotype structure (higher Wall's B and Wall's Q)
Relaxed Selection	Cheating behaviors do not experience strong selection, possibly	↑	↑	Allele frequency skew closer to zero (Tajima's D=0)

	because the multicellular (social) stage is rare in nature			
Purifying Selection	Cheaters are selected against, for example if relatedness is high	↓	↓	Excess of low frequency alleles (negative Tajima's D)

1
2