

unsatisfactory for both systems, because a likelihood ratio test strongly favors the model with migration limitation in both cases [for Amazon:  $\chi^2 = 24.6$ , 1 degree of freedom (d.f.),  $P < 0.001$ ; for CFR:  $\chi^2 = 19.4$ , 1 d.f.,  $P < 0.001$ ].

Estimates of  $\Theta$  can be converted to estimates of per capita speciation rate if it is possible to estimate the number of individuals in the metacommunity. With a much larger area, the Amazon rain forest metacommunity almost certainly contains at least as many individuals as does fynbos, which covers  $<50,000$  km<sup>2</sup>. The high estimates for  $\Theta$  thus imply a substantially higher per capita speciation rate in the CFR. These results are consistent with the prevailing view that the western CFR is an extremely migration-limited system with extraordinarily high speciation rates. Each of the local communities in our data set is an identifiable subregion of the CFR that consists of a range of hills or mountains and adjacent lowlands. Thus, the CFR metacommunity is topographically fragmented, which predicts low migration rates, consistent with our results. With these low migration rates, it is likely that local communities will be sufficiently isolated to allow ecological drift to cause divergence among communities, because few individuals per generation will be exchanged (19). Further, populations of individual species will be genetically isolated, so that genetic drift will tend to cause divergence and the formation of

new species. Thus, our results support the view that the fynbos metacommunity consists of topographical islands isolated not by water but by drier lowlands. This pattern contrasts sharply with that found in tropical rain forests, which exhibit high connectivity over long distances. Within the CFR, the association of the highest values of  $\Theta$ , and thus speciation rates, with the lowest migration rates supports the view that isolation over short spatial scales (1 to 100 km) has played a role in generating, as well as structuring, the high diversity of the CFR.

#### References and Notes

- H. P. Linder, C. R. Hardy, *Philos. Trans. R. Soc. London Ser. B* **359**, 1623 (2004).
- P. Goldblatt, J. Manning, *Cape Plants: A Conspectus of the Cape Flora of South Africa* (National Botanical Institute of South Africa, Cape Town, 2000).
- J. E. Richardson *et al.*, *Nature* **412**, 181 (2001).
- S. P. Hubbell, *The Unified Neutral Theory of Biodiversity and Biogeography* (Princeton Univ. Press, Princeton, 2001).
- R. M. Cowling, P. W. Rundel, B. B. Lamont, M. K. Arroyo, M. Arianoutsou, *Trends Ecol. Evol.* **11**, 362 (1996).
- P. Slingsby, W. J. Bond, *S. Afr. J. Bot.* **51**, 30 (1985).
- D. J. McDonald, J. M. Juritz, R. M. Cowling, W. J. Knottensbelt, *Plant Syst. Evol.* **195**, 137 (1995).
- R. H. Whittaker, *Taxon* **21**, 213 (1972).
- H. ter Steege *et al.*, *Biodiversity Conserv.* **12**, 2255 (2003).
- N. C. A. Pitman, J. W. Terborgh, M. R. Silman, P. V. Nuñez, *Ecology* **80**, 2651 (1999).
- N. C. A. Pitman *et al.*, *Ecology* **82**, 2101 (2001).
- O. J. Hardy, B. Sonké, *For. Ecol. Manage.* **197**, 191 (2004).
- R. Condit *et al.*, *Science* **295**, 666 (2002).
- J. Chave, *Ecol. Lett.* **7**, 241 (2004).
- I. Volkov, J. R. Banavar, S. P. Hubbell, A. Maritan, *Nature* **424**, 1035 (2003).
- D. Alonso, A. J. McKane, *Ecol. Lett.* **7**, 901 (2004).

- B. J. McGill, *Nature* **422**, 881 (2003).
- R. M. Cowling, Ed., *The Ecology of Fynbos: Nutrients, Fire and Diversity* (Oxford Univ. Press, Cape Town, 1992).
- C. Boucher, *Bothalia* **12**, 455 (1978).
- H. C. Taylor, *Cederberg Vegetation and Flora* (National Botanical Institute, Cape Town, 1996).
- B. E. Van Wyk, P. A. Novellie, C. M. Van Wyk, *Bothalia* **18**, 211 (1988).
- M. J. A. Werger, *Bothalia* **11**, 309 (1974).
- Materials and methods are available as supporting material on Science Online.
- R. M. Cowling, B. B. Lamont, *Aust. J. Bot.* **46**, 335 (1998).
- R. M. Cowling, P. M. Holmes, *Biol. J. Linn. Soc.* **47**, 367 (1992).
- A. Chao, R. L. Chazdon, R. K. Colwell, T.-J. Shen, *Ecol. Lett.* **8**, 148 (2005).
- R. M. Cowling, S. Proches, in *Plant Diversity and Complexity Patterns. Local, Regional and Global Dimensions*, H. Balslev, Ed. (The Royal Danish Academy of Sciences and Letters, Copenhagen, 2005), vol. 55, pp. 273–288.
- R. M. Cowling, A. T. Lombard, *Diversity Distr.* **8**, 163 (2002).
- Supported by NSF grant DEB008901 to J.A.S. and R.M.C. and by an NSF Graduate Research Fellowship to A.M.L. We are grateful to L. Mucina and S. Proches for assistance with CFR data sets, N. Pitman for providing Amazon data, P. Holmes Rebelo for vegetation survey data, and A. Rebelo and the Protea Atlas Project of the South African National Biodiversity Institute for interpretation. I. Koltracht provided code for numerical integration and B. McGill, R. Colwell, P. Linder, and three anonymous reviewers provided helpful comments.

#### Supporting Online Material

[www.sciencemag.org/cgi/content/full/309/5741/1722/DC1](http://www.sciencemag.org/cgi/content/full/309/5741/1722/DC1)

Materials and Methods  
References and Notes

1 June 2005; accepted 28 July 2005  
10.1126/science.1115576

## Generating Electricity While Walking with Loads

Lawrence C. Rome,<sup>1,2\*</sup> Louis Flynn,<sup>1</sup> Evan M. Goldman,<sup>1</sup>  
Taeseung D. Yoo<sup>1</sup>

We have developed the suspended-load backpack, which converts mechanical energy from the vertical movement of carried loads (weighing 20 to 38 kilograms) to electricity during normal walking [generating up to 7.4 watts, or a 300-fold increase over previous shoe devices (20 milliwatts)]. Unexpectedly, little extra metabolic energy (as compared to that expended carrying a rigid backpack) is required during electricity generation. This is probably due to a compensatory change in gait or loading regime, which reduces the metabolic power required for walking. This electricity generation can help give field scientists, explorers, and disaster-relief workers freedom from the heavy weight of replacement batteries and thereby extend their ability to operate in remote areas.

Over the past century, humans have become increasingly dependent on technology, particularly electronic devices. During the past decade, electronic devices have become more mobile, enabling people to use medical, communica-

tion, and Global Positioning System (GPS) devices as they move around cities or in the wilderness. At present, all of these devices are powered by batteries, which have a limited energy storage capacity and add considerable weight. Although substantial progress has been made in reducing the power requirements of devices and increasing the power densities of batteries, there has not been a breakthrough in the parallel development of a portable and renewable human-driven energy source (1, 2).

The combination of limited energy and the large weight of batteries poses the most critical problem for individuals having high electricity demands in remote areas and who are already carrying heavy loads (such as field scientists or explorers on prolonged expeditions). At present, replacement batteries may make up a substantial proportion (as much as 25%) of the very heavy packs ( $>36$  kg or 80 lbs) that such users must carry (3). To help solve this problem, we developed a passive device, the suspended-load backpack, which extracts mechanical energy from the vertical movement of the load during walking and converts it to electricity for powering portable devices.

During terrestrial locomotion, the environment does no work on the body (except for the small force of aerodynamic drag) and conversely, humans do no work on the environment. Rather, almost all of the mechanical work is generated and dissipated inside the body (4, 5). This makes it exceedingly difficult to capture mechanical energy to drive an electrical energy conversion apparatus, because the device would need to be either surgically placed within the body or attached to the outside of the body (such as an exoskeleton), which would affect the person's maneuverability and comfort. Therefore, researchers in the field have focused on putting devices in the only acces-

<sup>1</sup>Department of Biology, University of Pennsylvania, Philadelphia, PA 19104, USA. <sup>2</sup>Marine Biological Laboratory, Woods Hole, MA 02543, USA.

\*To whom correspondence should be addressed. E-mail: lrome@sas.upenn.edu

sible location: the shoe. Such “heel-strike” devices, however, have permitted only small levels of electrical energy generation (10 to 20 mW) (2, 6). The primary reason for this limitation is that on a hard surface, essentially no mechanical work (force times distance) is done at the foot/ground contact point, because under normal circumstances the point of vertical force application does not move in the vertical plane (that is, distance = ~0).

Although one can make the shoe compliant so that the foot moves a small distance because of compression of the sole and heel (7), this is problematic because increasing compliance leads to declining maneuverability and stability. Although considerable effort has gone into developing exotic energy-generating technologies for shoe devices (8), the small magnitude of the mechanical energy source remains a limitation.

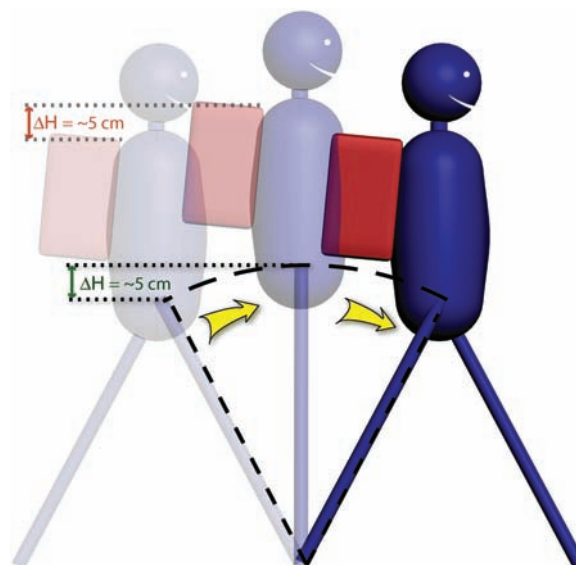
We recognized that the vertical movement of a heavy load in the gravitational field during walking represents a heretofore untapped source of mechanical energy and a potential opportunity to generate substantial levels of electricity. During walking, a person moves like an inverted pendulum (4, 5, 9): One foot is put down and then the body vaults over it, causing the hip to move up and down by 4 to 7 cm (10) (Fig. 1). Thus, if one is carrying a load in a backpack, because it is fixed to the body, it has to go up and down the same vertical distance (Fig. 1). A considerable amount of mechanical energy must be transferred (or generated de novo by the muscles) if the load is heavy. In the case of a 36-kg load, 18 J of mechanical energy transfer (or work) accompanies each step (assuming 5 cm displacement), and at two steps  $s^{-1}$ , this is equivalent to 35 W. Although this represents a large potential source of mechanical energy, it is also inaccessible if the load is rigidly attached to the body. We reasoned that decoupling the load from the body would allow the differential movement (between the load and the body) necessary for mechanical energy extraction and ultimately electricity production. We therefore designed a device, the suspended-load backpack (Fig. 2), that could be interposed between the body and the load, resulting in differential movement (Fig. 2) and the potential for generating a considerable amount of electrical energy.

Figure 3 shows the displacement and electrical output from the generator of a person walking with a 38-kg load (11). In this trial, the relative movement of the load with respect to the pack frame was approximately 4.5 cm (top panel). The linear velocity of the rack, in turn, drove the generator (a 25:1 geared dc motor) up to ~5000 rpm. The middle panel shows the voltage output of the generator. In these experiments, the output of the generator ran through a fixed “load” resistor (25 ohms), and hence the electrical power, calculated as  $voltage^2/resistance$ , is shown in the bottom panel. The

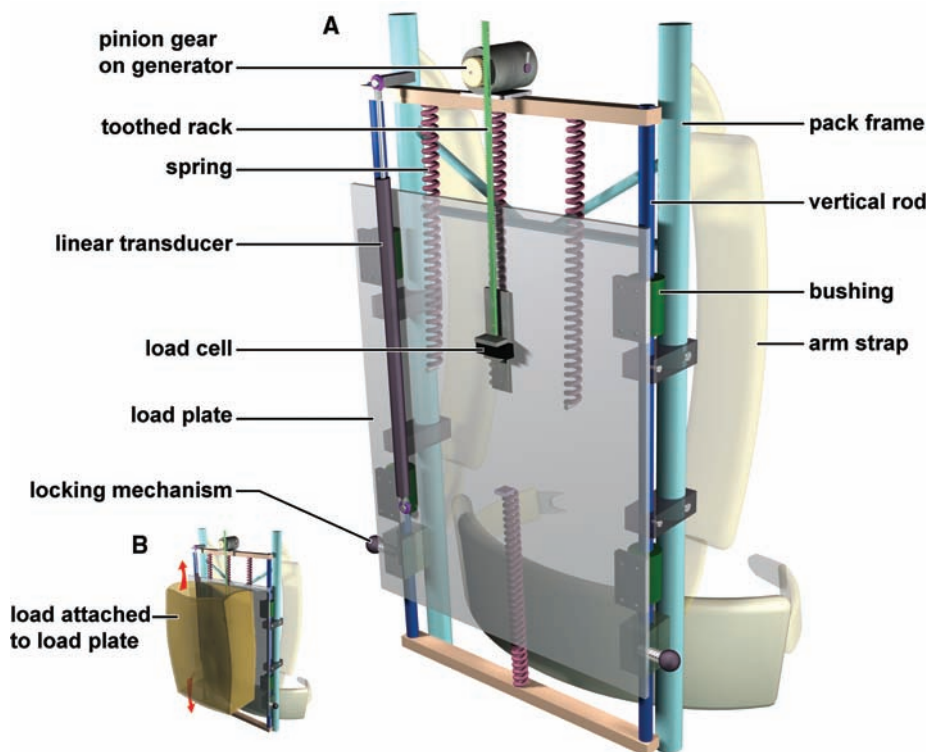
average electrical power in this trace is 5.6 W. This determination of electrical power was confirmed by joule heating experiments (11).

Six male participants walked at speeds ranging from of 4.0 to 6.4  $km\ hour^{-1}$  (2.5 to 4.0 mph) while carrying 20-, 29-, and 38-kg loads in addition to the fixed portion of the instrumented pack frame, which weighed 5.6 kg (12). Average electrical power increased

with walking speed and generally increased with the weight of the load in the pack (Fig. 4). Further, while walking up a 10% incline, electrical power generation for a given load and speed was equal to or greater than that on the flat (13). The maximum electrical power output obtained on the flat was 7.37 W ( $\pm SE = 0.49$ ,  $n = 6$  participants), or about 300 times higher than previously published values gen-



**Fig. 1.** Humans use an inverted pendulum mode of walking, in which the hip traces out an arc over an extended leg with a vertical excursion ( $\Delta H$ ) of approximately 5 cm. A backpack load rigidly attached to the body would undergo the same vertical excursion. This excursion drives electricity generation.

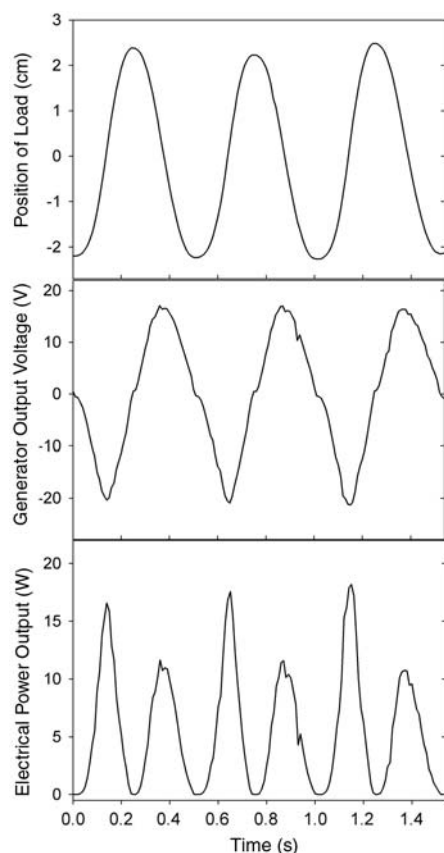


**Fig. 2.** In the suspended-load backpack, the pack frame is fixed to the body, but the load, mounted on the load plate, is suspended by springs (red) from the frame (blue) (A). During walking, the load is free to ride up and down on bushings constrained to vertical rods (B) (11). Electricity generation was accomplished by attaching a toothed rack to the load plate, which when moving up and down during walking, meshed with a pinion gear mounted on a geared dc motor, functioning as a generator, rigidly attached to the backpack frame.

erated from shoe devices (10 to 20 mW) (2, 6). The mechanical power removed by the generator (and gears) is the product of the average force exerted on the rack ( $F_{\text{rack}}$ ), the displacement of the load with respect to the pack frame ( $dl_{\text{rack}}$ ) (11), and the step frequency. Mechanical power into the generator increased with speed and load in a similar fashion as electrical power output. Hence, the efficiency of conversion of mechanical energy to electrical energy (that is, electrical power output divided by mechanical power input) was nearly constant (30 to 40%) over this range of speeds and loads.

To power portable devices (or charge batteries), the alternating polarity of the voltage and current (Fig. 3) must be rectified, which the suspended-load backpack can accomplish with little reduction ( $\sim 5\%$ ) in electrical power output (fig. S1) (11). Hence, using circuitry for voltage smoothing, the suspended-load backpack can power multiple devices such as cell phones or GPS receivers, both of which use less than 1 W (11).

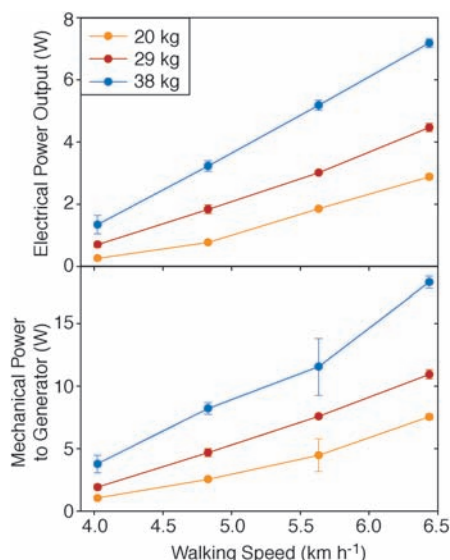
If generating electricity while wearing the backpack markedly increased metabolic rate, the device would be of limited use. Indeed, one would expect that because mechanical energy is



**Fig. 3.** Position, generator voltage, and electrical power output during walking. In these records, the person is walking with a 38-kg load at 5.6 km hour<sup>-1</sup>. “Position” refers to the position of the load with respect to the backpack frame, with the midpoint labeled as 0 cm.

continuously removed from the system by the generator, the muscles would need to perform additional mechanical work during electricity generation in order to replace it. For instance, the mechanical power input to the generator is 12.15 W while walking at 5.6 km hour<sup>-1</sup> and carrying a 29-kg load (table S3). Because the maximum efficiency of mechanical power production by human muscle is about 25% (14, 15), if the body movement was otherwise the same, one might anticipate a minimum increase of 48.6 W in metabolic power input. We measured the rate of O<sub>2</sub> consumption ( $\dot{V}O_2$ ) and CO<sub>2</sub> production ( $\dot{V}CO_2$ ) of participants walking with the backpack in two configurations: locked (no relative movement, mechanical energy loss, nor electrical energy generation) and unlocked (normal relative movement and electricity generation) in a repeated, paired protocol (11) specifically designed to resolve small differences. We found that the metabolic rate increase ( $\Delta$ metabolic power input) compared to that with the locked backpack was only about 19.1 W (table S1) (11), which is much less than would be predicted, providing an “apparent efficiency” of mechanical work production of  $\sim 63\%$  (table S3).

On the one hand, these results indicate that electricity can be generated metabolically more cheaply than anticipated. But on the other hand, they suggest that there must be some change in gait or loading regime while walking with the unlocked backpack, which causes a reduction of 29.5 W (48.6 minus 19.1 W) or about 3/5 of the metabolic power required for doing work against the generator. Considerable savings in metabolic cost have been previously reported in African women carrying loads on their heads



**Fig. 4.** The electrical power output and mechanical power input of the generator as a function of walking speed and load. This graph shows average values of four separate trials for each of 12 conditions for one person. The standard deviation bars are shown but are often smaller than the symbol.

and attributed to more efficient transfer between kinetic and potential energy (16). Although the precise mechanism of compensation in our study remains a mystery, an initial kinematic analysis revealed significant alterations in the biomechanics of walking that could be at the root of the reduction in metabolism. In particular, although there was no change in step frequency, the averaged vertical displacement of the hip during each step was 67.4 mm for the locked condition but only about 55.5 mm, or 11.9 mm less, for the unlocked condition (table S3). Further, there was an 11.8% ( $\pm$ SE = 1.67%,  $n = 4$  participants,  $P = 0.008$ ) reduction in the peak force exerted by the load back onto the person, as well as a change in phasing of this force with respect to the gait cycle (fig. S2). Because these factors will affect the magnitude and time course of forces, as well as the position of the center of mass, they will likely affect the amount of positive work that must be performed during the “double-support phase,” a major determinant of the cost of walking (17–21).

Finally, despite the smaller than predicted  $\Delta$ metabolic power input, individuals may have to carry extra food in order to power electricity generation. This weight, however, is negligible compared to the weight of batteries required to generate the same electrical energy. The specific energy of food ( $3.9 \times 10^7$  J kg<sup>-1</sup>) (22) is about 100-fold greater than the specific energy of lithium batteries ( $4.1 \times 10^5$  J kg<sup>-1</sup>) (1) and 35-fold greater than that of zinc-air batteries ( $1.1 \times 10^6$  J kg<sup>-1</sup>). Given that the “metabolic efficiency of electricity generation” (electricity power output/ $\Delta$ metabolic power input) is 19.5% (table S3), the extra food to be used for generating electricity would require about 20- and 6.8-fold less weight than lithium and zinc-air batteries, respectively (23). Hence, the longer the expedition, the greater the weight savings (24). Further, the  $\sim 12\%$  reduction in peak force exerted on the body by a given load (fig. S2), as well as the potential for using some of the extracted mechanical energy directly for cooling the user (such as through forced air ventilation with a fan or pumping of a coolant) would provide additional ergonomic benefits to the user.

Throughout history, humans have solved many problems by inventing passive devices to enhance the movements made by their muscles (such as springy bamboo poles to carry loads or skis to move through the snow) (25, 26). The suspended-load backpack is another passive device that may help solve a growing problem in the 21st century.

#### References and Notes

1. J. L. Gonzalez, A. Rubio, F. Moll, *Int. J. Soc. Mater. Eng. Resour.* **10**, 34 (2002).
2. J. Kyriassis, C. Kendall, J. Paradiso, N. Gershenfeld, in *IEEE International Conference on Wearable Computing* (IEEE Computer Society Press, Los Alamitos, CA, 1998), pp. 132–139.

3. S. Georgi, [www.batteriesdigest.com/id380.htm](http://www.batteriesdigest.com/id380.htm) (accessed June 2005).
4. R. M. Alexander, *J. Exp. Biol.* **160**, 55 (1991).
5. G. A. Cavagna, N. C. Heglund, C. R. Taylor, *Am. J. Physiol.* **233**, R243 (1977).
6. J. Drake, *Wired* **9**, 90 (2001).
7. S. Stanford, R. Pelrine, R. Kornbluh, Q. Pei, in *Proceedings of the 13th International Symposium on Unmanned Untethered Submersible Technology* (Autonomous Undersea Systems Institute, Lee, NH, 2003).
8. T. Starner, J. Paradiso, in *Low Power Electronics Design* (CRC Press, Boca Raton, FL, 2004), p. 45–1.
9. G. A. Cavagna, M. Kaneko, *J. Physiol.* **268**, 647 (1977).
10. S. A. Gard, S. C. Miff, A. D. Kuo, *Hum. Mov. Sci.* **22**, 597 (2004).
11. Supporting material is available on Science Online.
12. Because it is a prototype, there has been no attempt to reduce the weight of the backpack—indeed, it is substantially “overdesigned.” Further, the 5.6 kg includes the weight of six load cells and one 25-cm-long transducer, each with accompanying brackets and cables, as well as other components that will not be present on a typical pack. In future prototypes, we estimate that the weight will exceed that of a normal backpack by no more than 1 to 1.5 kg.
13. Under high-power conditions (5.6 km hour<sup>-1</sup> with 20- and 29-kg loads and 4.8 km hour<sup>-1</sup> with a 38-kg load), power generation on the incline was the same as on the flat. Under low-power conditions (4.8 km hour<sup>-1</sup> with 20- and 28-kg loads), electricity generation on the incline was actually substantially greater than that on the flat (table S1).
14. R. Margaria, *Biomechanics and Energetics of Muscular Exercise* (Clarendon, Oxford, 1976).
15. R. A. Ferguson *et al.*, *J. Physiol.* **536**, 261 (2001).
16. G. A. Cavagna, P. A. Willems, M. A. Legramandi, N. C. Heglund, *J. Exp. Biol.* **205**, 3413 (2002).
17. A. Grabowski, C. T. Farley, R. Kram, *J. Appl. Physiol.* **98**, 579 (2005).
18. J. M. Donelan, R. Kram, A. D. Kuo, *J. Exp. Biol.* **205**, 3717 (2002).
19. J. M. Donelan, R. Kram, A. D. Kuo, *J. Biomech.* **35**, 117 (2002).
20. J. S. Gottschall, R. Kram, *J. Appl. Physiol.* **94**, 1766 (2003).
21. Because this savings in metabolic energy represents only 6% of the net energetic cost of walking with the backpack (492 W) (table S3) (17, 18), accurate determinations of the position and movements of the center of mass, as well as the direction and magnitude of the ground reaction forces, are essential to discern the mechanism. This will require twin-force-platform single-leg measurements, as well as a complete kinematics and mechanical energy analysis (19, 20). The energy analysis is made more complex because the position of the load with respect to the backpack frame and the amount of energy stored in the backpack springs vary during the gait cycle. Finally, electromyogram measurements are also important to test whether a change in effective muscle moment arms may have caused a change in the volume of activated muscle and hence a change in metabolic cost (20, 27, 28).
22. K. Schmidt-Nielsen, *Animal Physiology: Adaptation and Environment* (Cambridge Univ. Press, Cambridge, ed. 3, 1988).
23. This assumes that electronic devices are being powered in real time. If there were a power loss of 50% associated with storage (such as in batteries) and recovery of electrical energy, then these factors would be halved.
24. When not walking, the rack can be disengaged and the generator cranked by hand or by foot. Electrical powers of ~3 W are achievable by hand, and higher wattage can be achieved by using the leg to power it.
25. R. Kram, *J. Appl. Physiol.* **71**, 1119 (1991).
26. A. E. Minetti, *J. Exp. Biol.* **207**, 1265 (2004).
27. A. A. Biewener, C. T. Farley, T. J. Roberts, M. Temaner, *J. Appl. Physiol.* **97**, 2266 (2004).
28. T. M. Griffin, T. J. Roberts, R. Kram, *J. Appl. Physiol.* **95**, 172 (2003).
29. This work was supported by NIH grants AR46125 and AR38404. Some aspects of the project were supported by Office of Naval Research grant N000140310568 and a grant from the University of Pennsylvania Research Foundation. The authors thank Q. Zhang, H. Hofmann, W. Megill, and A. Dunham for helpful discussions; R. Sprague, E. Maxwell, R. Essner, L. Gazit, M. Yuhas, and J. Milligan for helping with the experimentation; and F. Letterio for machining the backpacks.

**Supporting Online Material**

[www.sciencemag.org/cgi/content/full/309/5741/1725/DC1](http://www.sciencemag.org/cgi/content/full/309/5741/1725/DC1)

Materials and Methods

SOM Text

Figs. S1 and S2

Tables S1 to S4

References

14 February 2005; accepted 25 July 2005

10.1126/science.1111063

# Accurate Multiplex Polony Sequencing of an Evolved Bacterial Genome

Jay Shendure,<sup>1\*</sup> Gregory J. Porreca,<sup>1\*†</sup> Nikos B. Reppas,<sup>1</sup> Xiaoxia Lin,<sup>1</sup> John P. McCutcheon,<sup>2,3</sup> Abraham M. Rosenbaum,<sup>1</sup> Michael D. Wang,<sup>1</sup> Kun Zhang,<sup>1</sup> Robi D. Mitra,<sup>2</sup> George M. Church<sup>1</sup>

We describe a DNA sequencing technology in which a commonly available, inexpensive epifluorescence microscope is converted to rapid nonelectrophoretic DNA sequencing automation. We apply this technology to resequence an evolved strain of *Escherichia coli* at less than one error per million consensus bases. A cell-free, mate-paired library provided single DNA molecules that were amplified in parallel to 1-micrometer beads by emulsion polymerase chain reaction. Millions of beads were immobilized in a polyacrylamide gel and subjected to automated cycles of sequencing by ligation and four-color imaging. Cost per base was roughly one-ninth as much as that of conventional sequencing. Our protocols were implemented with off-the-shelf instrumentation and reagents.

The ubiquity and longevity of Sanger sequencing (1) are remarkable. Analogous to semiconductors, measures of cost and production have followed exponential trends (2). High-throughput centers generate data at a speed of 20 raw bases per instrument-second and a cost of \$1.00 per raw kilobase. Nonetheless, optimizations of elec-

trophoretic methods may be reaching their limits. Meeting the challenge of the \$1000 human genome requires a paradigm shift in our underlying approach to the DNA polymer (3).

Cyclic array methods, an attractive class of alternative technologies, are “multiplex” in that they leverage a single reagent volume to enzymatically manipulate thousands to millions of immobilized DNA features in parallel. Reads are built up over successive cycles of imaging-based data acquisition. Beyond this common thread, these technologies diversify in a panoply of ways: single-molecule versus multimolecule features, ordered versus disordered arrays, sequencing biochemistry,

scale of miniaturization, etc. (3). Innovative proof-of-concept experiments have been reported, but are generally limited in terms of throughput, feature density, and library complexity (4–9). A range of practical and technical hurdles separate these test systems from competing with conventional sequencing on genomic-scale applications.

Our approach to developing a more mature alternative was guided by several considerations. (i) An integrated sequencing pipeline includes library construction, template amplification, and DNA sequencing. We therefore sought compatible protocols that multiplexed each step to an equivalent order of magnitude. (ii) As more genomes are sequenced de novo, demand will likely shift toward genomic resequencing; e.g., to look at variation between individuals. For resequencing, consensus accuracy increases in importance relative to read length because a read need only be long enough to correctly position it on a reference genome. However, a consensus accuracy of 99.99%, i.e., the Bermuda standard, would still result in hundreds of errors in a microbial genome and hundreds of thousands of errors in a mammalian genome. To avoid unacceptable numbers of false-positives, a consensus error rate of  $1 \times 10^{-6}$  is a more reasonable standard for which to aim. (iii) We sought to develop sequencing chemistries compatible with conventional epifluorescence imaging. Diffraction-limited optics with charge-coupled device detection achieves an excellent balance because it not only provides submicrometer resolution and high sensitivity for rapid data acquisition, but is also inexpensive and easily implemented.

<sup>1</sup>Department of Genetics, Harvard Medical School, Boston, MA 02115, USA. <sup>2</sup>Department of Genetics, <sup>3</sup>Howard Hughes Medical Institute, Washington University, St. Louis, MO 63110, USA.

\*These authors contributed equally to this work.  
†To whom correspondence should be addressed.  
E-mail: shendure@alumni.princeton.edu (J.S.),  
gregory\_porreca@student.hms.harvard.edu (G.J.P.)

## Online Supplement

### Material and Methods

#### Kinematic measurements

Subjects walked on a Trackmaster treadmill at speeds ranging from 4.0 to 6.4 km h<sup>-1</sup> (2.5 to 4.0 mph). Movements in 3-D space were measured with a 6-camera Vicon 460 (Vicon Motion Systems Ltd., Oxford UK) system which determines 3-D positions of markers in its field of view. Subjects wore spherical reflective markers on the frame of the backpack at the level of the hip (attachment of hip belt). Using a custom routine in MATLAB, we analyzed approximately 10,000 individual steps during walking in the locked and unlocked conditions. We determined both the vertical excursion at the hip (measured by the height change of the reflective marker placed on the pack frame at the level of the hip belt) and the step frequency (Table S4). Two additional markers were attached to the frame of the pack and on the load. From these measurements we could determine the vertical and horizontal movement of the subject's hip, the frame of the backpack, and the load.

#### Instrumentation of the backpack

To determine the mechanical power input into the gears and generator (Dynetic Systems, 25:1 gear DC servo motor), or conversely, the mechanical power being removed from the system by the generator, we measured the force on the rack along its direction of movement and the displacement of the rack with respect to the frame. The rack force was measured with a load cell (Transducer Technologies MLP 100-C), through which it was attached to the moving load-plate (Fig. 2). The length change was measured by a linear potentiometer (CLP-200 Celesco Transducer Products, Chatsworth, CA). The analog output of each device was digitized and

stored along with the kinematic data using the Vicon A/D System. Each spring was also instrumented with a load cell and forces were recorded and used to determine the force exerted by the load back onto the backpack frame.

### **Electrical power measurements**

The electrical power production was determined from the voltage across a high wattage (50 Watt, Dale Power Resistor), 20 or 25 ohm resistor. The instantaneous power was calculated as  $\text{Voltage}^2/\text{Resistance}$  and the average power was determined by averaging the instantaneous power with time. The voltage was acquired by the Vicon system, but because of its high magnitude (up to 15-20 V), it was attenuated 10.4 times by a custom amplifier circuit which brought it below the +/- 10 V limit of the 16-bit A/D converter. This power measurement was verified by Joule heating experiments in which a waterproof 20 ohm load resistor was placed in an insulated, stirred volume of water fitted with a thermocouple. The rate of rise of temperature was used to calculate the power dissipated by the resistor. This value was in agreement with simultaneous measurements of electrical power described above. In experiments where the power output of the generator with the rectifier in place was compared to that without the rectifier, power output of the generator was taken as the product of the current (measured through a 0.1 ohm resistor placed before the rectifier) and voltage output of the generator.

### **Oxygen consumption and carbon dioxide production measurements**

The rate of oxygen consumption ( $\dot{V}O_2$ ) and carbon dioxide production ( $\dot{V}CO_2$ ) was measured with a breath-by-breath Parvomedics TrueOne system (Salt Lake City, Utah). This system's estimated repeatability is specified by the manufacturer at ~1%. As we were interested in average  $\dot{V}O_2$  rates, the system was programmed to average  $\dot{V}O_2$  over 1 minute time intervals.

The system analyzers were calibrated with a gas consisting of a 16% O<sub>2</sub> and 1% CO<sub>2</sub> mixture. The volume flow rate was measured with an installed pneumatac which was calibrated by pushing gas through it at different rates utilizing a 3 L syringe. Metabolic rate was calculated in Watts by the following formula: Metabolic power input (W) = 16.58 [W\*s/(ml O<sub>2</sub>)] \*  $\dot{V}O_2$  (ml/s) + 4.51 [W\*s/(ml CO<sub>2</sub>)] \*  $\dot{V}CO_2$ (ml/s) based on (SI).

### **Experimental protocol**

For determination of electrical and mechanical power output as a function of walking speed and load, subjects walked on the treadmill for several minutes for each measurement. Subjects performed each of the 12 load/speed tests (3 loads x 4 speeds) a total of 3 or 4 times. It should be noted that in preliminary experiments, the springs, gears, and load resistors were varied to try to maximize electricity production for a particular subject at a given walking speed and load. Because the spring and resistor choices were not finely graded, this represents only a rough optimization. These conditions were used throughout the later experiments.

Preliminary experiments showed that differences in the  $\dot{V}O_2$  between the unlocked and the locked backpack were likely to be very small (< 5%) and might be missed without careful experimental design. Hence during each day's experiments, a regimented protocol was adopted which permitted us to resolve these small differences. After a 7 minute warm-up period of walking with the pack, the subject walked for an additional 42 continuous minutes over which he was tested. The subject would walk with the backpack either locked or unlocked for a 7 minute trial, then switch to the other condition, and then switch back again for a total of six times. After each switch, the first 3 minutes of a trial were discarded, and the remaining 4 minutes were averaged. For each subject the unlocked and the locked values were calculated as the mean from

2 or 3 trials.

The protocol was sufficiently complex and time consuming for the subjects, that we limited our analysis to one load (29 kg) and one speed, 5.6 km h<sup>-1</sup> (3.5 mph). This represents a fast walking speed which generated a large level of electrical and mechanical power, and could be maintained for long periods.

### **Statistics and experimental design**

Preliminary experiments revealed that there was variation between individuals in electricity power generation and mechanical power loss which could be attributed to differences in gait (mainly differences in vertical excursion of the hip). Hence, wherever possible, we performed paired t-tests to assess differences between conditions. The level of significance was set at the 0.05 level. All statistics were performed using SigmaStat software v3.0.

### **Text**

#### **Behavior of Suspended-load Backpack without electricity generating technology**

With our initial prototype, which did not contain electricity generating technology, we found that normal walking induced very large vertical oscillations of the load. This suggested that considerable mechanical energy could be removed from the system (and converted to electrical energy) without over-damping. This fact gave us reason to suspect that the Suspended-load Backpack would permit far greater electrical power generation than could be generated by heel-strike devices.

#### **Walking on the incline**

When walking up an incline, we hypothesized that the user's hip may not undergo the same vertical excursions as during level walking, which might limit electricity production and hence



the usefulness of the device. We found, however, that under low power conditions (low loads and slow speeds), the subjects actually generated more electrical power walking on an incline than walking on a flat (Table S1). At higher load/speed conditions, there were no significant differences. We did not perform these measurements at the highest walking speed ( $6.4 \text{ km h}^{-1}$ ) as the mechanical work against gravity for a 78 kg subject carrying a 38 kg load would represent an additional 212 W.

### **Emergent property of backpack system leads to lower net power losses upon rectification.**

Because of the high output voltage of the generator compared to the diode drops associated with the rectifying circuit, we expected a relatively small (10-20%) power loss upon rectification. However, we found it was much lower than expected, only about 5%. It appears that with the rectifier in place, the electrical power output of the generator increased, thus compensating for a portion of the loss in the rectifier. For six subjects (four from the original experiments and two additional ones) measured at  $5.6 \text{ km hr}^{-1}$  with a 29 kg load, the generator electrical power output increased by 8.3 % ( $\pm \text{SE}=0.73$ ), the loss in the rectifier was 13.1% ( $\pm \text{SE}=0.65$ ), thus reducing the net loss to only 4.9% ( $\pm \text{SE}=0.48$ ). This effect can be reproduced by using an actuator to drive the backpack, showing that much of this effect is a property of the backpack itself. Figure S1 shows that with the rectifier in place, the displacement of the load is larger (5.9%). This led to higher revolution velocity of the generator and 10.9% greater average power output from the generator. Subtracting the 11% loss in the rectifier, the net loss was only 0.3%.

Although detailed mechanical modeling is necessary to verify the precise mechanism, the actuator measurements provide a likely possibility. With the rectifier in place, generator current and power output are “clamped” at zero (current flow prevented by diodes when voltage is less

than the diode drop) for about 7 ms (panel F) as the load changes directions (panel E). By contrast, the power is zero for only about 1 ms when the rectifier is not in place. The extra time with reduced damping appears to allow the load to accelerate more rapidly and to undergo a larger vertical excursion, which in turn permits greater power generation at later parts of the cycle. It should be noted, however, that during periods when the backpack with the rectifier is generating more electrical power, it is likely that the damping is larger as well.

### **Powering portable devices**

The backpack generates sufficient power for many devices. For instance, the simultaneous use of an MP3 player, PDA, night vision goggles (or 3 LED headlamp), handheld GPS, CMOS image decoder, GSM terminal in talk mode, and Bluetooth would take less than 2 W (Table S2). Further, wearable computers with relatively high performance require only 640 mW at 221 MHz.

Although the power requirements of most devices continue to decrease, off-the-shelf laptop computers and some communication devices still require more power than our generator can produce in real time. Nevertheless, these devices can be powered by stored energy.

Ultracapacitors can store several hours of energy with relatively little loss and higher energy density storage can be achieved with batteries, though they later incur a 30-50% loss in energy during charging and usage.

### **Alternate energy sources**

Absent a universal energy generation system, different technologies satisfy different user niches. For example, electricity generation at a stationary field site with predictably high ambient light levels may be best achieved by a light-weight 10 W solar panel. It should be noted, however, that maximum ratings are based on 1000 W/m<sup>2</sup> irradiance and power output is directly

proportional to irradiance level. Hence, with an irradiance of  $200 \text{ W/m}^2$ , power output would only be about  $\sim 2 \text{ W}$ . Recently solar panels have been added to backpacks. In addition to limitations associated with irradiance level, the angle of the panel with respect to the sun, which cannot be easily controlled while walking along a path, can, according to the manufacturer (Voltaic), cause the wattage to be reduced by an additional 80-90% (i.e., for the time the panel is angled away from sun).

For the above reasons, the Suspended-load Backpack will be superior for users already on the move with significant loads, particularly in lower ambient light environments (e.g., forested, overcast, or nighttime conditions, or non-optimal incidence angle of the sun). Further, the substitution of more efficient generators may permit considerably higher electrical power outputs with a given load, as well as the capability of generating the same power levels as reported here while carrying lighter loads. Ultimately, utilizing multiple technologies would provide greater flexibility to workers in remote areas.

## Figure Legends

**Figure S1.** Emergent property of backpacks leads to reduced net power loss with rectification.

Voltage (A) and power (B) across the load, power output of the motor (C), and displacement of the load with respect to the backpack frame (D) are shown for the rectifier in place (red traces) and rectifier out (black traces). With the rectifier in place, the excursion is greater (D), leading to a larger power output of the motor (C) such that the net power output across the load (B) is nearly unchanged. One possible explanation of the increased power output is that with the rectifier in place, the electrical power output of the generator is “clamped” at zero for about 7 ms (F) at the load turn around point (E) compared to only about 1ms when the rectifier is not in place. This in turn may underlie the faster acceleration and larger excursions of the load (E) which permits greater power generation. Note that panels E and F show an expanded timescale and ordinate. Note also that different traces were obtained with different acquisition hardware acquiring at different speeds. Because the traces were synchronized manually, there may be up to 5 ms error in the phasing.

**Figure S2.** Forces exerted by the load on the body are reduced in the Suspended-load Backpack.

The figure shows the summed forces exerted on the load during walking in the locked (black) and unlocked (red) conditions overlaid on the same graph. The subject is walking at  $5.6 \text{ km h}^{-1}$  with a 29 kg load. In the locked condition, an instrumented locking bar keeps the load from moving along the axis of the frame and measures the force. In the unlocked condition, this bar is removed and the forces from the 5 instrumented springs and the instrumented rack are summed. Note that higher peak forces are generated for a shorter time in the locked condition whereas a lower, broader peak is observed in the unlocked condition. There is also a difference in phase

with respect to the hip movements (not shown). The reduced forces may be responsible for three of the four subjects rating the unlocked backpack being more comfortable than the locked one, and the fourth rating it slightly less comfortable. However, there were not a sufficient number of subjects to demonstrate significance (Wilcoxon Signed Rank Test,  $P=0.25$ ,  $N=4$ ).

Figure S1 part 1

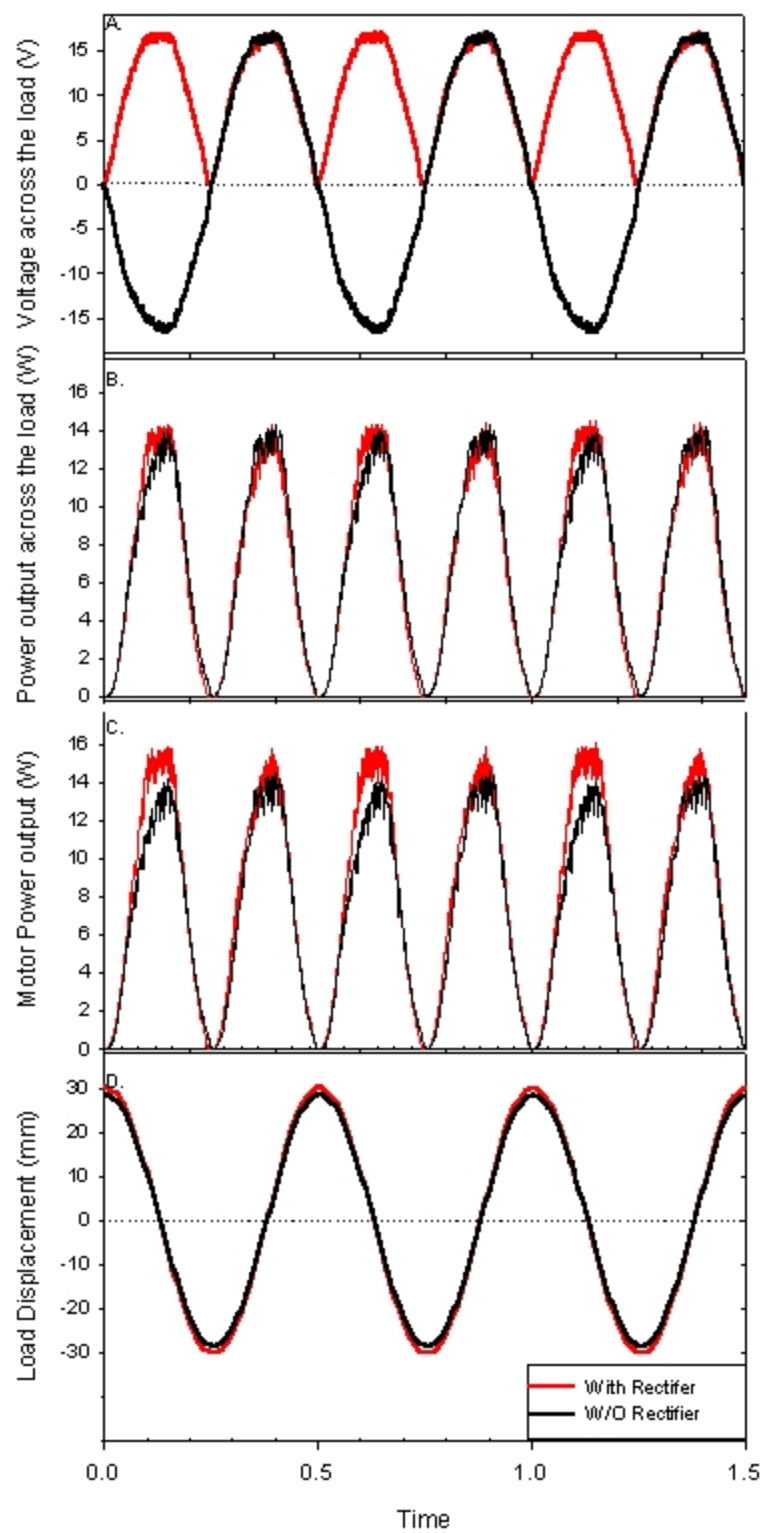


Figure S1 part 2

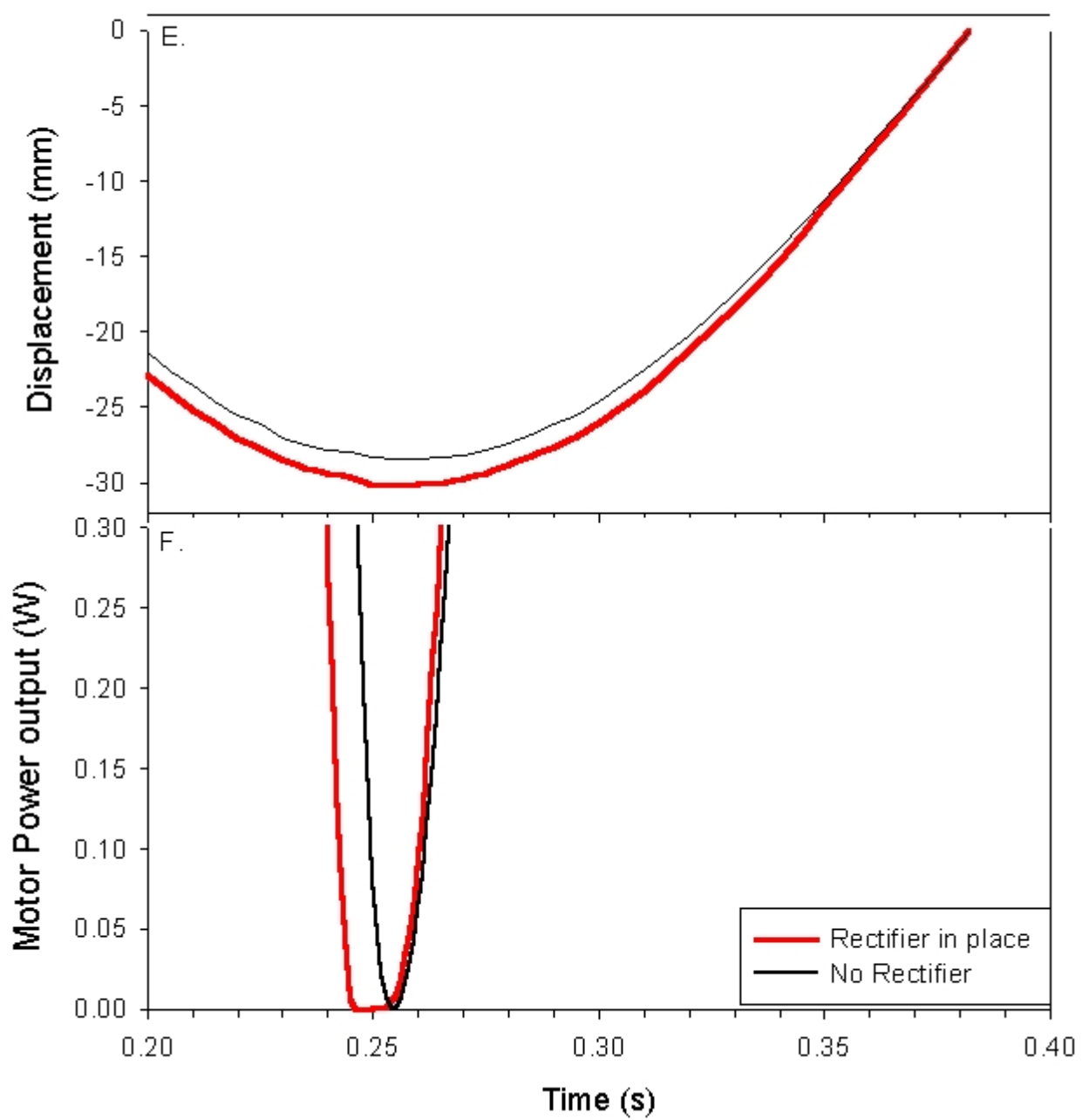
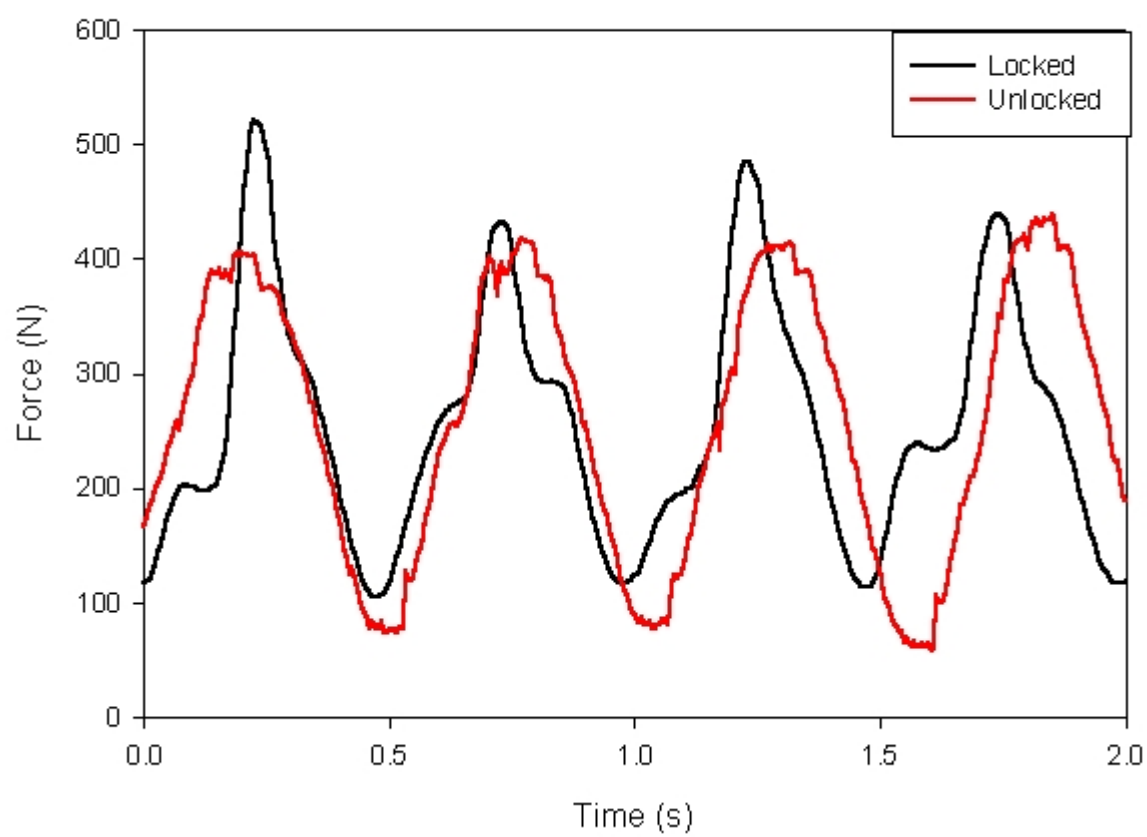


Figure S2





Load (kg),	20	28	38	20	28
Speed (km h <sup>-1</sup> )	4.8	4.8	4.8	5.6	5.6
Incline	3.11 (0.62)	3.49 (0.69)	3.89 (0.96)	3.27 (0.88)	3.98 (0.52)
Flat	1.82 (0.69)	2.42 (0.42)	3.79 (0.63)	3.26 (0.73)	3.96 (0.53)
Difference	1.29 (0.28)*	1.52 (0.44)*	0.11 (0.78)	0.01 (0.56)	0.02 (0.40)

**Table S1.** The average electrical power output (W) from four of the six original subjects walking on the flat and on a 10% incline at five load/speed conditions (note for the 28 kg at 5.6 km h<sup>-1</sup> conditions, two additional subjects were used-i.e., N=6). Each subject was tested at each condition three times. The SE is shown in parentheses. Asterisks denote significant differences (P<0.05) using paired t-tests. Note that under low power conditions (low speed and weight) walking on an incline actually increased power output whereas for higher power conditions there was no difference between walking on a flat and on an incline. We did not test the highest speed/load conditions because walking up a 10% incline at the fastest speed (6.4 km h<sup>-1</sup>) increases the mechanical work requirement by approximately 212 W (calculation assumes subject weighing 78 kg is carrying a 38 kg load).

Device	Power usage	Manufacturer, Model or Source
1. MP3 Player with headphones	120 mW	(S2)
2. PDA	50 mW	Palm III (S2)
3. Night Vision Goggle	60 mW	Pyser-SGI PNG-2+
4. IR Laser illuminator for Night Vision	200 mW	IZLID 425P Used when no ambient light
5. Handheld GPS	825 mW	Lowrance iFinder Go portable
6. Three-LED headlamp	600 mW	Petzl (measured)
7. CMOS image decoder	50 mW	(S2)
8. GSM terminal in talk mode	300 mW	(S2)
9. Bluetooth	50 mW	(S2)
10. Cell Phone	250-600 mW	Digital
11. Wearable computer (Linux Advanced Radio Terminal.)	106-640 mW	Power depends on adjustable clock speed 59-221 MHz (S3)
12. UV Water purification Device	5 W	SteriPen (45 s for 0.5 L)

**Table S2.** Power requirements for portable electronics.

Values are given for devices which might be powered by the Suspended-load Backpack. Note that the cumulative power requirement for items 1-9 is about 2W (using either night vision or a headlamp but not both). This value of power is easily attainable with the backpack. The manufacturer or information source is provided in the last column.

	Metabolic Unlocked (W)	Metabolic Locked (W)	Metabolic Difference (W)	Metabolic Difference (%)	Mechanical Power (W)	Electrical Power (W)	Conversion Efficiency (%)
	623	610	13.16	2.16	11.62	3.70	31.89
	652	614	37.93	6.18	17.63	5.26	29.83
	587	588	-1.19	-0.20	8.62	2.54	29.45
	615	592	22.82	3.86	17.31	4.73	27.31
	519	504	14.88	2.95	7.29	2.60	35.60
	640	614	26.81	4.37	10.44	3.44	32.92
Mean	606	587	19	3.22	12.15	3.71	31.17
SE	19.6	17.1	5.45	0.88	1.79	0.45	1.20

**Table S3.** Average metabolic, mechanical and electrical power and conversion efficiency

(electrical power output/mechanical power input) generated while carrying a load in the Suspended-load Backpack in the unlocked and locked conditions. N=6 subjects for all measurements. The metabolic rate in the unlocked and locked conditions are significantly different ( $P=0.017$ , paired t-test). On average the net efficiency of mechanical power production was 63% (mechanical power output/ $\Delta$ metabolic input) and the net efficiency of electrical power production (power output/ $\Delta$ metabolic input) was 19.5%. Note also that the mechanical power for the 29 kg load is significantly larger than shown in Figure 4 (7.5 W). Fig. 4 shows data from only one individual who had a lower mechanical power loss than the other subjects. Finally, we determined the cost of standing for our 6 subjects to be 114 W ( $\pm$  SE= 6.5) so that we could determine the net metabolic cost of walking with the load (i.e., walking metabolic rate-standing, ( $S4$ ,  $S5$ )). The net cost of walking was 492 W ( $\pm$ SE= 18.8).

	Hip Excurs Unlocked (mm)	Hip Excurs Locked (mm)	Hip Excurs Difference (mm)	Step Freq Unlocked (Hz)	Step Freq Locked (Hz)	Step Freq Difference (Hz)
	47.5	53.3	-5.83	2.119	2.117	0.001
	63.6	84.0	-20.4	1.954	1.936	0.018
	50.7	58.1	-7.38	1.876	1.892	-0.015
	62.8	75.8	-13.0	1.922	1.917	0.005
	54.3	70.2	-15.9	1.989	1.966	0.024
	54.2	63.1	-8.94	1.984	1.941	0.043
Mean	55.50	67.42	-11.91	1.974	1.962	0.012
SE	2.64	4.68	2.28	0.03	0.03	0.008

**Table S4.** Kinematic changes while walking with a 29 kg load. 468-798 steps were analyzed for each subject under each condition. Average values are shown for each of the six subjects. A paired *t*-test showed that the unlocked case had a significantly lower excursion ( $P = 0.003$ ). There was no significant difference in step frequency ( $P = 0.20$ ).

## References

- S1. J. M. Brockway, *Hum. Nutr. Clin. Nutr.* **41**, 463 (1987).
- S2. J. L. Gonzalez, A. Rubio, F. Moll, *Int. J. Soc. Mater. Eng. Resour.* **10**, 34 (2002).
- S3. J. D. Bakker, K. Langendoen, H. Sips, (*IEEE 21st International Conference on Distributed Computing Systems Workshops (ICDCSW '01)*, 2001).
- S4. A. Grabowski, C. T. Farley, R. Kram, *J Appl. Physiol.* **98**, 579 (2005).
- S5. J. M. Donelan, R. Kram, A. D. Kuo, *J. Exp. Biol.* **205**, 3717 (2002).