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A Selective Advantage to Immigrant Genes in a Daphnia Metapopulation

Dieter Ebert, *et al.*

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long-term electricity harvesting in open systems such as sedimentary environments.

Microbial communities consisting of fermentative microorganisms and Geobacteraceae are capable of degrading complex assemblages of organic matter (*22*). Thus, it seems likely that Geobacteraceae could aid in the harvesting of energy not only from aquatic sediments and subsurface environments, but also from a wide variety of organic waste materials. Molecular studies have shown that microorganisms in the Geobacteraceae are important members of the microbial community involved in the anaerobic degradation of aromatic hydrocarbons in petroleum-contaminated aquifers (*23, 24*), but this metabolism is often limited by the availability of Fe(III) (*25*). It may be possible to simply use electrodes to increase the electron-accepting capacity of contaminated sediments and enhance bioremediation of the subsurface. Further study of the mechanisms for electron transfer from the Geobacteraceae to electrodes should aid in designing strategies to optimize these processes.

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5. Electrodes were rinsed under a stream of sterile marine buffer to remove all visible sediment and debris, then scraped with sterile razor blades to remove adherent bacteria. DNA was extracted from this graphite slurry by a standard lysozyme-SDS–hexadecyltrimethyl ammonium bromide (CTAB) phenol-chloroform method and further cleaned using a Wizard DNA purification kit (Promega). DNA was independently extracted from electrodes used in four different incubations.
6. To reduce PCR bias, we amplified 16*S* rDNA genes in two different reaction mixtures, using the bacterial primers 27F and 519R or 63F and 519R. The products of these reactions were pooled before cloning with a TOPO TA cloning kit. The first 500 base pairs of cloned 16*S* genes were sequenced to determine phylogenetic placement. Results shown in (*7*) are from a single set of electrodes; libraries from other incubations gave similar results.
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13. A dual-chambered fuel cell was constructed using 54-mm OD (outside diameter) glass tubing and a 22-mm OD pinch clamp assembly. Electrodes used in dual-chamber fuel cells had a surface area of 25.4 cm^2. The volume of each chamber was 225 ml. The chambers were separated by a cation-selective membrane (Nafion 117).
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15. The aerobic chamber of a two-chambered cell was filled with an aerobic marine buffer; the anaerobic chamber was filled with *Desulfuromonas* growth medium lacking electron donor or acceptor. Common electron shuttles were avoided by omitting resazurin and cysteine, and by culturing cells using fumarate as the electron acceptor. The headspace of the anaerobic chamber was flushed with oxygen-free N_2:CO_2 (80:20). All fuel cells were maintained at 30°C. Current and voltage measurements were collected with a multimeter.
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17. For poised-potential growth, both chambers were filled with anaerobic *Desulfuromonas* (marine) or *Geobacter* (freshwater) growth medium lacking electron donors or acceptors. Resazurin and cysteine were omitted from the medium. Both chambers were maintained under oxygen-free N_2:CO_2 (80:20). The reference electrode was a standard 3M NaCl Ag/AgCl electrode. Potential at the working electrode was maintained at +200 mV versus the reference electrode by a potentiostat interfaced with a Macintosh computer, which also recorded current flowing to the working electrode.
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A Selective Advantage to Immigrant Genes in a *Daphnia* Metapopulation

Dieter Ebert,[1,2,3]*† Christoph Haag,[1,2,3]* Mark Kirkpatrick,[4] Myriam Riek,[1,2] Jürgen W. Hottinger,[1,2,3] V. Ilmari Pajunen[2,5]

Immigrants to habitats occupied by conspecific organisms are usually expected to be competitively inferior, because residents may be locally adapted. If residents are inbred, however, mating between immigrants and residents results in offspring that may enjoy a fitness advantage from hybrid vigor. We demonstrate this effect experimentally in a natural *Daphnia* metapopulation in which genetic bottlenecks and local inbreeding are common. We estimate that in this metapopulation, hybrid vigor amplifies the rate of gene flow several times more than would be predicted from the nominal migration rate. This can affect the persistence of local populations and the entire metapopulation.

Gene flow between populations can be both a creative and a constraining force in evolution (*1–3*). The introduction of new genetic material into a population increases local genetic diversity and helps the spread of favorable alleles across metapopulations. On the other hand, it reduces genetic variation between populations and hinders local adaptation. In inbred populations, the consequences of migration may be particularly important: If the hybrid offspring of immigrants and residents are competitively superior, their hybrid vigor will amplify the gene flow caused by migration (*4–6*). Furthermore, the demographic consequences of increased vigor could prevent the decline and even the extinction of populations (*7–12*). The magnitude of hybrid vigor is, however, controversial. Highly inbred populations may have low genetic loads because inbreeding exposes recessive deleterious alleles to purging by natural selection (*13–17*).

In subdivided populations with local extinctions and colonizations, genetic bottlenecks can be frequent, leading to increased homozygosity (*15*, *16*, *18*). If homozygosity results in a fitness reduction (inbreeding de-

[1]Zoologisches Institut, Universität Basel, Rheinsprung 9, 4051 Basel, Switzerland. [2]Tvärminne Zoological Station, SF-10900 Hanko, Finland. [3]Université de Fribourg, Département de Biologie, Unité d'Écologie et Évolution, Chemin du Musée 10, 1700 Fribourg, Switzerland. [4]Section of Integrative Biology, University of Texas, Austin, TX 78712, USA. [5]Division of Population Biology, Department of Ecology and Systematics, University of Helsinki, Post Office Box 17 (Arkadiankatu 7), SF-00014 University of Helsinki, Finland.

*These authors contributed equally to this work.
†To whom correspondence should be addressed. E-mail: dieter.ebert@unifr.ch

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pression), then small amounts of immigration can have disproportional effects on the vigor and persistence of local populations. This will in turn influence the extinction and colonization dynamics of the whole metapopulation (*4*–*6*).

To test whether hybrid vigor has an effect on the genetic structure of local populations, we studied the consequences of immigration in small populations of the water flea *Daphnia magna*. This planktonic crustacean occurs in various bodies of water, ranging from large ponds, with genetically diverse populations, to small intermittent pools, characterized by low genetic diversity. In our metapopulation, *D. magna* inhabits small pools (0.5 to 20 m^2 and 0.1 to 0.5 m deep) on the rocky banks of islands along the Swedish and Finnish Baltic Sea coast (*19*–*22*). The number of these rock pool populations is thought to be larger than 10^5, with one to a few hundred pools per island. In our study area around Tvärminne in southern Finland, local extinction rates are about 20% per year, estimated over an 18-year study including 507 pools (*21*, *23*). About 5% of the empty pools are colonized per year, and the proportion of occupied pools has remained around 20% since 1983. The life cycle of *D. magna* begins with the hatching of females from resting eggs in spring, followed by asexual reproduction for up to 12 generations. At the end of the season, sexual reproduction produces resting eggs that survive the winter and also serve as dispersal stages.

In 1998, a population genetic survey of 96 *D. magna* populations with known age indicated that local populations are often founded by a single clone, followed by a population increase due to clonal expansion (*24*). Such genetic bottlenecks lead to substantial inbreeding during sexual reproduction, which is most extreme when a single founder clone produces resting eggs by mating with clonal brothers and sisters (known as selfing) (*25*). The frequent occurrence of genetic bottlenecks makes this metapopulation an ideal site to study the impact of local inbreeding on the success of immigrants.

To test whether hybrid vigor occurs in this metapopulation, we introduced immigrant genotypes into 22 *D. magna* populations and monitored the fate of their outbred offspring over one summer season. We chose 22 rock pool populations on nine islands in July 1999. These rock pools were known to be continuously inhabited by *D. magna* for at least 2 years before the beginning of the study. Of the five genetic loci known to be polymorphic in these metapopulations, the 22 studied populations were polymorphic at none (12 populations), one (six populations), or two loci (four populations), which is typical of the metapopulation. In July 1999, we removed all *D. magna* from these populations and kept 200 to 500 females from each population with minimal selection in the laboratory. To remove resting eggs that may have been produced earlier in that season, we removed the water and most of the soft sediments from the pools. Comparable disturbances occur naturally in this metapopulation, where storms on the Baltic Sea frequently wash out parts of or even entire pools (*19*, *21*). We also collected 22 clones from different pools within the study area and propagated them in the laboratory. These we designated as our experimental immigrant clones.

Two weeks later, after rain had refilled the pools, we brought 200 individuals of the populations back into their original pools and added 200 individuals of one immigrant clone into each pool (*26*). In each experimental pool, the immigrant clone differed from the local clones at at least one allozyme marker locus. To avoid bias due to potential fitness effects of the genetic markers, we used multiple alleles at four loci. The genetic markers allowed us to distinguish between hybrids, offspring of local residents, and selfed offspring of the immigrant clone after the populations had undergone one round of sexual recombination, i.e., in spring of the following year.

Rock pools were left undisturbed until we took the first samples in May 2000. From these samples, we genotyped 66 to 122 animals and also founded laboratory populations using 200 to 300 females each. This was done to duplicate the natural rock pool experiment under controlled laboratory conditions so that we could decouple the effect of inbreeding from uncontrolled environmental effects, such as the local pool environment, predators, and further immigrants from the same or other *Daphnia* species (*27*). Second and third samples were taken from all populations about 60 and 100 days after the first samples had been collected.

Our hypothesis was that the outbred offspring would increase in frequency as the result of hybrid vigor. Figure 1 shows that this was the case in all rock pool populations where we recovered hybrids in May 2000 (all but pool 15). It was also the case in 17 of 18 laboratory populations (Fig. 1) (*28*). The changes in genotype frequencies in the field and the laboratory were highly correlated with each other ($r = 0.71$ to 0.93 for the three offspring types, $P < 0.002$, $n = 16$), and the increase of outbred genotypes did not differ significantly among them (paired t test: $t = 0.52$, $P = 0.61$, $n = 16$). Hybrids also increased significantly in the time period between the second and the third sample ($P < 0.01$ in the field and the laboratory) at a time when genotypes with very low fitness (e.g., due to castrating homozygous recessive mutations) had already been purged. Therefore, the observed hybrid vigor is at least partially due to deleterious effects of alleles with weak to intermediate effect (*6*, *29*). As selection against such alleles is weak, they can accumulate to high frequencies. High genetic

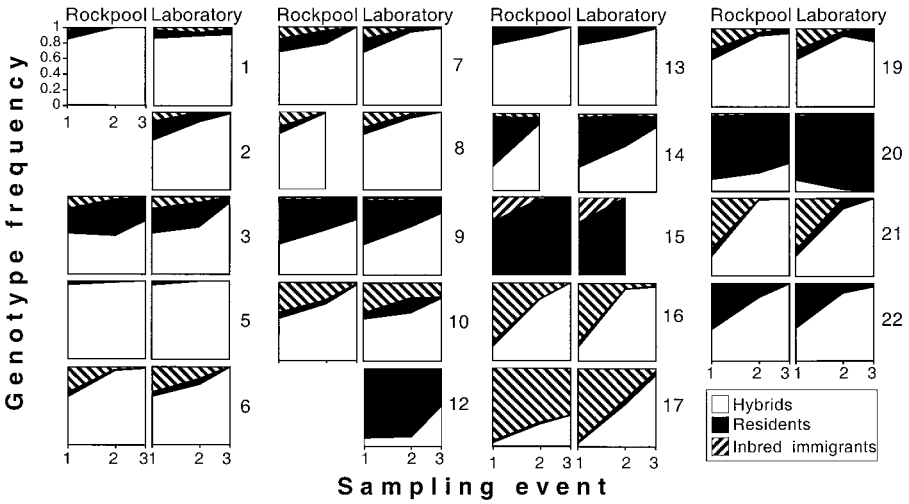


Fig. 1. Frequency changes of hybrids, residents, and inbred immigrants during asexual competition. The left plot of each pair of area plots shows frequency changes in the natural rock pools, whereas the right plots show changes in the laboratory. The population numbers are given on the right. All plots have the same *y* axis (genotype frequencies ranging from 0 to 1) and *x* axis (sampling events at 0, 60, and 100 days). The first sampling date was between 18 May and 5 June 2000 (depending on the emergence of *Daphnia* from resting eggs), the second between 18 and 21 July 2000, and the third on 28 August 2000. Empty places for two of the rock pool area plots and area plots including only the first and the second sample indicate extinction of these populations after the first or the second sample, respectively. Only 19 of the 22 populations are shown, because populations 4 and 11 went extinct during the winter and in population 18 only residents were found (sample size >300). No hybrids were detected in population 15 and no inbred immigrants in populations 12, 13, and 22. In all other populations, we found all three offspring types.

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loads have been reported for *Daphnia* (*30*–*32*).

We believe that hybrid vigor best explains our results, as a number of alternative hypotheses can be excluded. First, because we used a different immigrant clone for each population, it is unlikely that all of them carried superior alleles (*33*). Second, although rare genotypes could have had an advantage by, for example, exploiting unoccupied niches, we rejected this hypothesis because hybrids increased in frequency irrespective of their initial frequencies in May 2000 (Fig. 1). Third, although residents may suffer from locally adapted predators or parasites (*34*, *35*), predators were excluded from the laboratory, and the increase of hybrids did not differ significantly between populations infected with a parasite or not (*36*). Fourth, mating success cannot explain our results (*37*–*39*) as hybrid success was measured during the asexual phase of the *Daphnia* life cycle.

One key effect of hybrid vigor is an increase in effective gene flow (*4*). We estimate that 2 years after a natural immigration event (where immigrants represent a much smaller fraction of the population than in our experiments), the effective rate of gene flow is about 35 times larger than would be predicted by the number of immigrants alone, and it will even increase further in the following years (*40*). The amount by which gene flow is amplified depends on the causes and magnitude of hybrid vigor. It may be much smaller in genetically more diverse *Daphnia* populations but may be high in other systems characterized by frequent extinctions and recolonizations.

Amplified gene flow caused by hybrid vigor may account for lower observed levels of population differentiation than predicted by models based on neutral effects (*4*, *6*, *41*–*43*) and may influence the evolution of dispersal (*44*). An important effect of hybrid vigor is the "genetic rescue" of populations from extinction, because it may influence extinction and colonization dynamics of the whole metapopulation (*5*). Although we did not show a link between population persistence and inbreeding depression, such a relation has been shown before (*9*, *11*, *12*) and is likely to apply to *Daphnia* metapopulations as well. In this light, gene flow is an essential component for the persistence of metapopulations. Thus, our study gives clear empirical support for the need to maintain gene flow in the management and conservation of subdivided populations.

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24. In 1998, we genotyped 22 to 44 *D. magna* from all 96 pools (out of 507) occupied in the study region at five polymorphic loci using allozyme electrophoresis (*45*). Twenty-nine independent (i.e., pools not connected to other pools) new colonizations were recorded in this summer, none of which was polymorphic at more than two loci. In 26 of these populations, only one genotype was found. Of these, five were fixed at a heterozygote locus, suggesting that they may have been colonized by only one clone each. One population was composed of two heterozygote clones (genotypes AA/Bb and Aa/BB) with strong linkage disequilibrium ($P < 0.01$), suggesting that it may have been colonized by two polymorphic clones. The finding of five fixed heterozygotes among 29 newly colonized populations is in contrast to the finding of only one fixed heterozygotes among the 67 older populations (colonized in 1997 or earlier) that underwent at least one season of sexual reproduction ($P = 0.009$, Fisher exact test). At least three of the 29 new populations resulted from interisland migration, because no other *D. magna* populations were present on these islands during previous years.
25. With selfing, the inbreeding coefficient F of the entire population will be at least 0.50 in the following year. As resting eggs usually come in pairs, founding events may include two clones that are related to each other as full or half sibs. Then F in the following year would be at least 0.25 or 0.125, respectively.
26. Under natural conditions, the number of immigrants is likely to be very small relative to the number of residents. To minimize stochastic events influencing the fate of the immigrants during our experiment, we introduced the local residents and the immigrants in equal numbers.
27. Laboratory populations were kept in white buckets filled with 8 liters of artificial medium. Populations were kept at 18°C and 18:6 hours light:dark cycle and were fed daily with 1.5×10^7 cells of *Scenedesmus gracilis*. Five liters of the medium was exchanged after 6 and 11 weeks. Population size was 250 to 400. Predators and competitors were excluded from these populations.
28. The genotype frequency differences between the last successful sample (third sample, unless extinction occurred after the second sample) and the first sample were tested with a Wilcoxon sign rank test (paired test). While the hybrids increased significantly (field: $Z = 68$, $P < 0.0001$, $n = 16$; laboratory: $Z = 82.5$, $P < 0.0001$, $n = 18$), the residents (field: $Z = -60$, $P < 0.0001$, $n = 16$; laboratory: $Z = -65.5$, $P = 0.0028$, $n = 18$) and the selfed immigrant offspring decreased significantly (field: $Z = -44.5$, $P = 0.0005$, $n = 15$; laboratory: $Z = -60$, $P < 0.0001$, $n = 16$). Tests excluded population 15, because no hybrids were found.
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40. The impact of hybrid vigor on gene flow can be quantified by the "effective migration rate" $m^*(t)$, defined as the migration rate that would have the same impact on the genetic composition of the population as an equivalent number of copies of a migrant gene t years after it arrives if hybrid vigor was absent. The following argument applies to selected as well as neutral genes. In the *Daphnia* metapopulation, migrants arrive as resting eggs before the growing season of year 1 or as asexual females during it. They form clones during year 1 that mate with residents to produce F_1 hybrid resting eggs. At the end of year 1, $m^*(1) = m$ because hybrid vigor is not yet expressed. If the number of immigrants is small relative to residents and *Daphnia* mate randomly or disassortatively, then virtually all immigrants mate with residents. Thus, $m^*(2) = w_{F_1} m$, where w_{F_1} is the mean fitness of the hybrids relative to residents. From our data during the 100-day study period (representing about one sexual generation), we estimate $m^*(2) = 33.7m$ using the laboratory data and $36.0m$ using the field data. The fitness of hybrids was estimated as

$$w_{F1} = [(1 - p_1)p_3]/[p_1(1 - p_3)]$$

where p_1 and p_3 are the frequencies of the hybrids at the beginning and end of the experiment (about one sexual generation). As replicates in which $p_3 = 1$ (hybrids went to fixation) were not included, our estimates of w_{F1} are underestimates. In subsequent years, m^* can be determined for genes that are unlinked to other loci under selection: $m^*(t) = w_{F1}w_{B(1)}w_{B(2)} \ldots w_{B(t-2)}m$, where $w_{B(1)}$ is the mean fitness of individuals resulting from the first backcross between the F_1 and residents and $W_{B(i)}$ is the mean fitness of offspring from backcross $i - 1$ and residents. This expression for m^* is an approximation that holds so long as the frequency of matings between backcross individuals is negligible in all years. The value of $m^*(t)$ will grow in successive years if the backcrosses have higher fitness than the residents, reaching an asymptote as the population reaches linkage equilibrium. We do not have the fitnesses of the backcrosses, and so we calculate m^* using a simple model that assumes that fitness differences are caused by deleterious mutations of equal effect at unlinked loci. Assuming that the mutations have multiplicative effects, then the fitness of a genotype can be described by the numbers of homo- and heterozygous mutants that they carry. We write the relative fitness effects of a single locus as 1, $(1 - hs)$, and $(1 - s)$ for genotypes with 0, 1, and 2 copies of a mutation. Assume that migrants and residents each consist of a single clone that is entirely homozygous, with the migrants and residents fixed for alternative alleles at i_m and i_r, loci, respectively. Calculations then show that

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$$w_{F1} = \frac{(1 - hs)^{(i_m + i_r)}}{(1 - s)^{i_r}},$$

$$w_{B(1)} = (1 - \tfrac{1}{2}hs)^{i_m}\left[\frac{2 - s - hs}{2(1 - s)}\right]^{i_r}$$

and that the fitnesses of later backcrosses converge geometrically on 1 at a rate of 1/2 per sexual generation (year). Using parameter values suggested for *Daphnia* (*46*) ($s = 0.14$ and $h = 0.3$) and setting $i_m = i_r = 55$ to give an F_1 fitness similar to what we observed, we obtain $m^*(2) = 35.7m$, $m^*(3) = 234m$, and $m^*(\infty) = 6703m$. Lower values of m^*, but still substantially greater than 1, can be obtained when i_m and i_r have more modest values. Thus, hybrid vigor can make gene flow many times stronger than would be predicted from the nominal migration rate under these conditions.

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Systems Analysis of Ran Transport

Alicia E. Smith,[1] Boris M. Slepchenko,[2] James C. Schaff,[2] Leslie M. Loew,[2] Ian G. Macara[1]*

The separate components of nucleocytoplasmic transport have been well characterized, including the key regulatory role of Ran, a guanine nucleotide triphosphatase. However, the overall system behavior in intact cells is difficult to analyze because the dynamics of these components are interdependent. We used a combined experimental and computational approach to study Ran transport in vivo. The resulting model provides the first quantitative picture of Ran flux between the nuclear and cytoplasmic compartments in eukaryotic cells. The model predicts that the Ran exchange factor RCC1, and not the flux capacity of the nuclear pore complex (NPC), is the crucial regulator of steady-state flux across the NPC. Moreover, it provides the first estimate of the total in vivo flux (520 molecules per NPC per second and predicts that the transport system is robust.

In vitro studies of nucleocytoplasmic transport estimate a maximum rate of $\sim 10^3$ translocation events NPC^{-1} s^{-1} (*1*). In the intact cell, however, steady-state flux rates might be a fraction of this value. To determine what factors contribute most toward the overall regulation of flux, we needed to integrate at a systems level the data for all the independent transport steps. This process required computational analysis. Cargo is shuttled through the NPC as a cargo-carrier complex, and a lower limit for cargo flux can be approximated if the steady-state flux of Ran is known, because at least one molecule of Ran is translocated in each direction for each complete cycle of carrier and cargo transport. We therefore used experimental and computational methods to estimate the steady-state flux rate for Ran in intact cells and to identify factors that most contribute to the global regulation of Ran transport.

We constructed a mathematical model of the Ran transport system in a mammalian cell using a modeling program called Virtual Cell (*2*, *3*). Biochemical reactions were localized in two subcellular compartments (cytosol and the nucleus), and flux rates between the compartments were calculated as the product of a permeability constant and the concentration difference across the nuclear envelope, describing facilitated diffusion by the transport carriers. Passive diffusion of Ran through the NPC was also incorporated. For the Ran transport cycle, reported data constrained the parameter values of reversible binding interactions and enzyme-mediated reactions (*4*–*10*) [Web table 1 and Web fig. 1 (*11*)]. All simulation results were obtained with the same parameter set, except where explicitly indicated.

For visualization of Ran import into the intact cell, the recombinant protein was modified with a fluorescent maleimide and was determined by several criteria to be functional [Web fig. 2 (*11*)]. This tagged Ran species (FL-Ran) in the model allowed direct comparisons of the simulation and experimental results. FL-Ran was injected into the cytosol of BHK-21 cells and imaged (at 0.5-s intervals) until nuclear accumulation reached a steady state (Fig. 1A) (*12*). Nuclear fluorescence intensities were converted to units of concentration by comparison to known standards (*12*). Import occurred with single exponential kinetics and reached a steady state within 12 to 30 s (at 23°C) (Fig. 1B) [Web fig. 3A (*11*)].

We also used a mutant species [Ran(T24N)], which is defective in binding to nuclear transport factor 2 (NTF2), the Ran

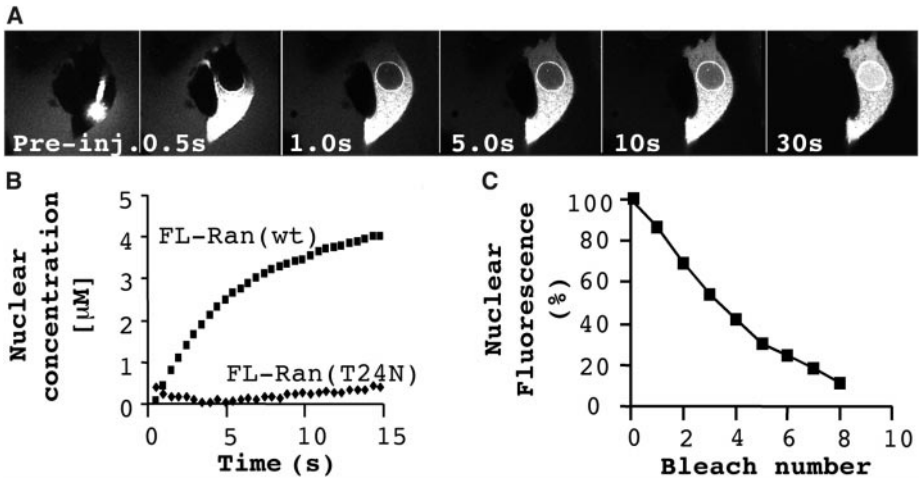


Fig. 1. Nucleocytoplasmic transport of FL-Ran in vivo. (**A**) Nuclear accumulation of FL-Ran in BHK-21 cells after cytosolic injection. (**B**) Comparison of time courses of nuclear accumulation for wild-type FL-Ran (initial cytosolic concentration = 7.8 μM) and T24N (initial cytosolic concentration = 9.0 μM). (**C**) Fluorescence loss in photobleaching on FL-Ran at steady state in microinjected BHK-21 cells. Graph shows remaining nuclear fluorescence intensity after each photobleaching cycle.

[1]Center for Cell Signaling, Departments of Pharmacology and Microbiology, University of Virginia, Charlottesville, VA 22908, USA. [2]Center for Biomedical Imaging Technology, University of Connecticut Health Center, Farmington, CT 06030, USA.

*To whom correspondence should be addressed. E-mail: igm9c@virginia.edu

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