

# Variation in blade morphology of the kelp *Eisenia arborea*: incipient speciation due to local water motion?

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**ABSTRACT:** The southern sea palm kelp *Eisenia arborea* produces wide, bullate (bumpy) blades in low-flow areas, whereas in adjacent high-flow areas blades are flat and narrow. Here we determine if morphological differences in these 2 closely associated populations are correlated with physical factors in the environment, and whether this response is a genetically fixed or plastic trait. Both phenotypes were subjected to morphometric analysis, field and laboratory transplant experiments, and genetic analysis (M13 DNA fingerprinting). Physical variables (water motion, temperature, and light) were monitored simultaneously in the field. Results indicate that the 2 populations, separated by <10 m, are genetically distinct and transplants in the field and lab remained similar to individuals from the original collection location, regardless of the imposed flow environment. Water motion varied significantly between the 2 populations and was the single physical variable that correlated with morphology. Based on bump heights and water velocities in the field, bullate blades could increase turbulent transport of nutrients 4-fold that of flat blades. The thicker blades and large holdfasts of the flat morph may impart higher survivorship under high-flow conditions. This suggests water motion selects for 2 distinct morphotypes of *E. arborea* that represent adaptations to enhance fitness under different nutrient availability and drag force regimes. Thus, blade morphology is not a plastic response to the local environment but a genetically fixed trait and may be indicative of nascent speciation in the face of strong selection by water motion over spatial scales smaller than observed previously in kelps.

**KEY WORDS:** Multilocus DNA fingerprinting · Population differentiation · Phenotypic plasticity · Water motion · Santa Catalina Island · Laminariales

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## INTRODUCTION

Morphological variation in response to local environmental conditions is common among many marine organisms (Gerard & Mann 1979, Druehl & Kemp 1982, West et al. 1993, Miller & Dorr 1994, Bruno & Edmunds 1997, Arsenault et al. 2001, Trussell & Etter 2001, Hill & Hill 2002). In many cases, morphological variation within a species has led to rapid genetic variation sufficient for population divergence and evo-

lution of new species (e.g. West-Eberhard 1989, Brazeau & Harvell 1994, Johannesson et al. 1995, Howard & Berlocher 1998, Miller et al. 2000, Wilson et al. 2000). Although kelps and other marine algae exhibit conspicuous morphological variation, the evolutionary consequences of this variation have rarely been studied (but see Miller et al. 2000). In kelps, for example, the giant kelp *Macrocystis pyrifera* produces thick, flat blades in areas exposed to large waves or strong currents, but thin blades with ruffled margins in sheltered

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areas (Druehl & Kemp 1982, Hurd et al. 1997). Similarly, the long-stemmed kelp *Laminaria longicuris* produces narrow and strap-like blades in exposed areas, but large and highly ruffled blades in protected areas (Gerard & Mann 1979). It is not known, however, whether the morphological variation observed in kelps is produced by genetic differentiation or is induced by the environment.

Several workers (Wheeler 1980, Koehl & Alberte 1988, Hurd & Stevens 1997, Hurd et al. 1997) have hypothesized that variation in blade morphology may be an adaptation to local water motion, with wide undulate blades dominant in low-flow areas. The undulations are thought to induce turbulence at velocities lower than that predicted for flat blades, thereby replenishing nutrient supplies more rapidly than if transport were by molecular diffusion alone. Basic hydrodynamic principles state that projections on a surface (i.e. bumps on blades) can increase turbulence and thereby enhance mixing and transport relative to a smooth, flat surface (Schlichting 1979). Conversely, flat streamlined blades have lower drag than large, ruffled blades (Denny 1988, Koehl & Alberte 1988) and may therefore impart higher survivorship to individuals with flat blades in high-flow areas.

In subtidal coastal areas off southern California, the southern sea palm kelp *Eisenia arborea* produces 2 distinct morphotypes that are correlated with the local flow environment. Preliminary observations indicated that *E. arborea* growing in low-flow areas produced blades that were wider and more bullate (bumpy) (Fig. 1), and that holdfasts produced fewer haptera than in individuals from high-flow areas. Are these morphological differences inducible responses to local water motion (or some other physical factor), or is morphology genetically fixed?

Here, we employed a multidisciplinary approach to examine the potential causative relationship between

environmental factors and the morphology of blades in *Eisenia arborea*, and how variation in morphology is related to selection. The close association of the 2 morphs in the field (<5 m in some cases) provides a unique opportunity to examine the impact of a single physical variable without the confounding effects of local site variability in biological (i.e. herbivory or competition) or physical factors (wave exposure, day length, etc.). First, we characterized the important physical variables experienced by each morphotype as well as the degree of morphological variation between and within morphotypes. Specifically, we examined (1) blade morphometrics in high-flow and low-flow populations in both exposed and sheltered areas, (2) water velocities associated with each blade morph, and (3) the correlation of blade morphology with other physical factors such as light, temperature, and nutrient concentrations. We then used these variables in a simple calculation of turbulence generated by the blade surface to predict whether morphology in *E. arborea* could indeed be adaptive in terms of mass transport.

Second, we examined the genetic relationship between the bullate and flat morphs using 4 different techniques: (1) multilocus DNA fingerprinting to quantify the degree of genetic differentiation between morphotypes of low- and high-flow populations separated by <10 m; (2) a reciprocal transplant study in which juveniles of each morphotype were exposed to both high- and low-flow conditions, and subsequently monitored for morphological changes; (3) a common garden study in which juveniles of each morphotype were exposed to a low-flow, high-nutrient environment and (4) comparison of the experimental sites to an exposed site located >40 km distant as a 'natural' experiment; to control for the potential effects of light and water motion at depth (i.e. if light were important, we would predict the presence of the low-flow morph at depth in exposed areas).

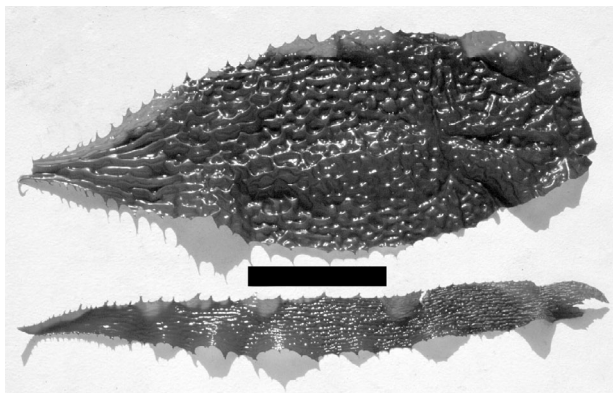


Fig. 1. *Eisenia arborea* blade morphs. Top: bullate blade from low-flow environment. Bottom: flat blade from high-flow environment. Scale bar = 16.2 cm

## MATERIALS AND METHODS

**Study areas.** Studies were conducted at Santa Catalina and San Clemente Islands in the Southern California Bight (Fig. 2). The bight offers protection from northern and southerly swells for northeastward-facing beaches on the islands, but southwestward-facing areas are directly exposed to large oceanic swells (Winant et al. 1999, NOAA buoy data, <http://seaboard.ndbc.noaa.gov>, station = 46026). Water motion is much reduced on the NE side of the islands, and in protected coves or deep areas (>20 m depth), water motion is practically zero, as indicated by the presence of silt and sedimentation, which dust the blades of

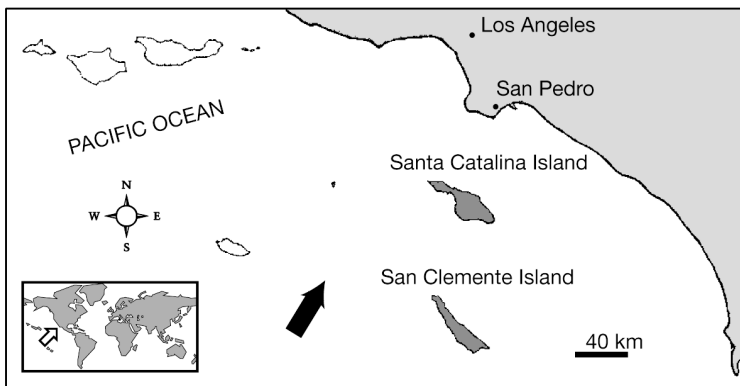


Fig. 2. Southern California Bight. Solid arrow indicates dominant swell direction (NOAA buoy data, Station ID 46026)

kelps in these areas throughout the year (L.M.R. pers. obs.). In this study, areas of highly reduced water motion are called low-flow areas. Areas with visible agitation by waves are termed high-flow, and areas facing southward exposed to oceanic swells are called exposed areas.

Due to the formation of a warm, counter-clockwise eddy in the bight, Santa Catalina and San Clemente Island experience the warmest seawater temperatures in California (Owen & Power 1980). The high temperatures (>20°C in the summer) support a unique and diverse flora and fauna, including many normally tropical species found nowhere else in California (Littler & Power 1980). The high temperatures also result in frequent local extinctions of kelp (North 1971, 1994, Gunnill 1985, Zimmerman & Robertson 1985, Dayton et al. 1999), as nutrient concentrations required for kelp growth are inversely related to water temperature (see 'Physical environment' below) and are often undetectable during the summer months (Carpenter & Capone 1983, Zimmerman & Kremer 1984, Kopczak et al. 1991).

**Morphometrics.** A total of 72 mature, undamaged blades were randomly collected from both low-flow and high-flow areas at Santa Catalina Island. High- and low-flow areas were selected based on 2 criteria: (1) the presence of either the bullate or flat morph and (2) local water velocities (see 'Field flow' below for description of water velocity measurements). Four characteristics were recorded for each blade: maximum length, width, thickness, and bump height (Fig. 3). To compare characters between populations, an ANOVA was performed separately on each character at a significance level of 0.05 and all ANOVA assumptions (normality, homogeneity of variances) were tested prior to analysis. All statistical analyses in the present study unless noted otherwise were performed using Statistica (StatSoft).

Bump heights were used to estimate the roughness Reynolds number ( $Re^*$ ), a non-dimensional measure of the degree of turbulence generated by a surface (Schlichting 1979).  $Re^*$  is defined as

$$Re^* = u^* h_s / \nu \quad (1)$$

where  $u^*$  = the friction velocity (here assumed to be 10% of the freestream velocity in  $\text{m s}^{-1}$ ),  $h_s$  = the roughness height (the average blade bump height in m), and  $\nu$  = the kinematic viscosity of seawater ( $\text{m}^2 \text{s}^{-1}$ ). A fully rough surface has a  $Re^* > 60$ , whereas a hydrodynamically smooth surface (i.e. the surface has roughness elements but they are small relative to the boundary layer thickness) falls in the range

$Re^* < 5$ . In between these 2 extremes, the transition region ( $5 < Re^* < 60$ ) describes flow where the influence of viscosity and turbulence are of the same order of magnitude. Bumpy blades with a  $Re^* > 5$  may have higher mixing rates (i.e. higher nutrient delivery) than blades with lower  $Re^*$  values.

In addition to the blade characteristics, surface areas of holdfasts (area attached to the substratum) were measured. Young juveniles (approx. 50 cm total length) were removed from the substratum, and the bases of the holdfasts were photographed using a digital camera. The surface areas of holdfasts were measured in these images using NIH Image (<http://www.winimage.com>).

To gain a better understanding of the relationship between water flow and blade morphology, morphological measurements were made along physical gradients between the 2 populations. The Bird Rock site at Santa Catalina Island (33° 26.808' N, 118° 29.105' W) has a vertical wall where water velocity, according to linear wave theory, is a function merely of depth (for a given

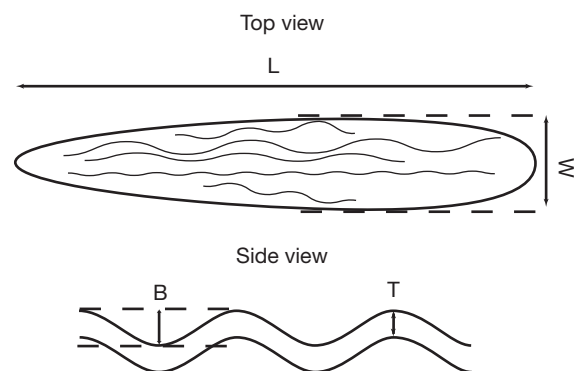


Fig. 3. Measurement of blade morphometrics. L = length, W = width, T = thickness, and B = bump height (measured as total blade thickness including bumps minus T)

wave height; Denny 1988). A vertical transect was made from 0 to 20 m at 2 locations on the wall and the morphometrics of blades were measured (as described above) on all individuals within 3 m of the transect at 0, 5, 10, 15, and 20 m depth. Holdfast morphology (creeping, dense, or unclear; holdfasts were usually one morph or the other instead of a continuum) also was noted. We define a creeping holdfast as one with few, long haptera and a dense holdfast as one with many, short haptera. Horizontal water velocities ( $u$ ) were predicted using the expression from linear wave theory for nearshore breaking waves (Denny 1988):

$$u = (\pi H/T)[\cosh(ks)/\sinh(kd)] \quad (2)$$

where  $H$  is wave height (in m),  $T$  is wave period (in s),  $k$  is the wave number ( $2\pi/L$ , where  $L$  = wave length in m),  $s$  is the distance above the bottom (in m), and  $d$  is the depth of water (in this case, 30 m). Velocities were calculated for  $s$  from 29 to 10 m (depth of 1 to 20 m from the surface) with wave conditions present during the summer months ( $H = 0.1$  m and  $T = 10$  s, NOAA buoy data described above and L.M.R. pers. obs.). A linear regression analysis was used on log-transformed data to characterize the relationship between morphology (blade width and bump height) and predicted velocity.

As low-flow areas were correlated with increased depth at the study sites on Santa Catalina Island, 20 blades were randomly collected at 20 to 25 m depth along with 20 blades from 5 m depth on the exposed SW face of adjacent San Clemente Island (exposed directly to oceanic swell; see Fig. 2) to control for potential effects of light on blade morphology. An additional 20 blades were collected from depths of 20 and 5 m at a protected site on the NE side of the island (a total of 80 blades from San Clemente). All blades were measured as described above. High- and low-flow areas on San Clemente Island were determined based on personal observations and on prevailing swell directions from the NOAA buoy data described above. Only bump heights were analyzed statistically using a 2-way ANOVA with site (Santa Catalina and San Clemente Island) and flow (high and low) as factors. The control for depth was analyzed separately using a 1-way ANOVA to compare the high-flow treatments by collection location (Catalina high-flow, San Clemente exposed shallow (5 m), and San Clemente exposed deep (20 m)).

**Physical environment.** Water velocities were measured at 2 Santa Catalina locations where blades were collected for the morphometric study: Bird Rock and Intake Pipes (33° 26.682' N, 118° 28.949' W). Water velocity measurements were made using a Marsh-McBirney electromagnetic flow meter in winter 1997 and summer and fall 1998. The probe was anchored 0.5 m off the bottom (to mimic the location of blades in the water

column) with a modified concrete block. Divers using SCUBA positioned the block within 1 m of adult *Eisenia arborea*.

Velocity was measured for 1 to 5 min at a frequency of 10 to 100 Hz and the data were recorded using a PC laptop computer with a NI PC516 A/D PCMCIA card (National Instruments). Due to the oscillatory nature of the flow (mean velocity is approximately zero), the standard deviation about the mean or the root mean square (RMS) velocity was used to determine the magnitude of flow experienced by individuals. The average and SD of the RMS velocity, as well as the maximum velocity per sampling period, were calculated over the different sites for each habitat type (22 high-flow, 24 low-flow samples). An ANOVA was used to test for differences between areas we have designated as high-flow and low-flow in both summer and winter at a significance level of 0.05 and all ANOVA assumptions were tested prior to analysis.

To compare velocities between exposed and protected areas, an S4 electromagnetic current meter (InterOcean) was deployed at Catalina Head on the exposed NW side of Santa Catalina Island (33° 25.368' N, 118° 30.706' W) at depths of 5 ('shallow') and 10 m ('deep') in July and August 2002. Water velocities were sampled at 2 Hz for 18 min every hour for 48 h (26 to 28 July) and 144 h (30 July to 6 August) for a total of 4 d shallow and 4 d deep. The average and SD of the RMS flow and the maximum velocity per sampling period were calculated for each depth.

Water temperature at all 4 locations was monitored every 5 min (3 mHz) from June 1998 to October 1999 using small (30 mm diameter) temperature loggers ('Tidbit,' Onset Computer) attached to small floats 0.5 m above the substratum. Averages, standard errors and dominant frequencies were calculated for each month. Historical temperature data from 1982 to 1993 were provided by the NOAA Catalina RDG buoy (station = 46026).

Due to the strong relationship between nutrients and temperature at Santa Catalina Island (Zimmerman & Kremer 1984), average monthly nitrate concentrations ( $[\text{NO}_3^-]$ ) were calculated using the relation for water temperature from Zimmerman & Kremer (1984):

$$<15^\circ\text{C}: [\text{NO}_3^-] = 60.2 - 3.9(^\circ\text{C}) \quad (3) \\ (R^2 = 0.81, p < 0.001)$$

and

$$>15^\circ\text{C}: [\text{NO}_3^-] = 3.0 - 0.1(^\circ\text{C}) \quad (4) \\ (R^2 = 0.27, p < 0.01)$$

where  $[\text{NO}_3^-] = \mu\text{M}$  nitrate and  $^\circ\text{C}$  = temperature values measured here. The average nitrate concentrations at the 4 experimental sites (Bird Rock high- and low-flow, Intake Pipes high- and low-flow) during the

critical summer–early fall months, when water temperatures are high and wave action is low (June to October), were analyzed using a 2-way ANOVA. Site (Bird Rock and Intake Pipes) and flow (high and low) were used as factors.

The amount of photosynthetically active radiation (PAR;  $\mu\text{mol quanta m}^{-2} \text{s}^{-1}$ ) available for photosynthesis from the surface to 20 m was measured using a  $2\pi$  cosine light sensor (Li-Cor). A total of 23 measurements were recorded every meter at midday for a total of 5 d in June and October 1998 and August 2002 at Bird Rock. Surface conditions on all but 2 d were sunny and clear. The light extinction coefficient ( $K_d$ ) characterizing the light penetration of the water column was calculated using the relation (Kirk 1983)

$$E_d(z) = E_d(0)e^{-K_d z} \quad (5)$$

where  $E_d(z)$  is the irradiance (PAR) at depth  $z$ ,  $E_d(0)$  is the irradiance immediately under the surface (depth of 0 m). A Quasi-Newton iterative estimation procedure was used to determine  $K_d$  for  $R^2$  fit greater than 0.9.  $K_d$  was calculated separately for the cloudy days.

**Genetic analysis.** Individuals were collected from populations in low-flow (15 to 23 m depth) and high-flow (3 to 8 m) environments that were separated by 5 to 10 m on the Bird Rock vertical wall. DNA was extracted from 2 to 3 g of meristematic tissue as previously described (Coyer et al. 1994). Average yield was  $6.4 \text{ mg DNA g}^{-1}$  fresh weight tissue ( $n = 36$ , 0.7 to 47.8 g). DNA quality was evaluated with 0.8% agarose gels.

Because the low- and high-flow populations were separated by <10 m, we examined genetic variability with a multilocus DNA fingerprinting technique and chose the M13 technique rather than the random amplified polymorphic DNA (RAPD) method. Recent work has demonstrated that M13 fingerprinting is more appropriate for resolution of genetic variability on a local-scale, whereas RAPD fingerprinting is superior for regional-scale analysis (Coyer et al. 1997).

The detailed protocol for M13 DNA fingerprinting is provided in Coyer et al. (1994). In brief, purified DNA was digested with *RsaI* and size-fractionated on a 0.8% agarose gel. DNA was transferred (under vacuum) from the gel to a nylon membrane (Hybond-N, Amersham) and hybridized with a 778-bp fragment of M13mp18 phage DNA that was non-radioactively labeled with digoxigenin-11-deoxyuridine triphosphate (Boehringer-Mannheim). Bands were visualized using alkaline phosphatase-conjugated anti-digoxigenin coupled with chemiluminescence (Lumi-Phos 530, Boehringer-Mannheim) and exposed to Kodak X-Omat film for 0.5 to 3.0 h. For most samples, at least 2 separate restriction enzyme digests were analyzed on at least 2 separate gels in order to evaluate the possibility of partial digests and to resolve questionable bands.

Sizes of bands and error estimates of molecular size measurements (e.g. determination of bin size) were determined using a computer-based imaging system (FragmeNT, Molecular Dynamics) (Coyer et al. 1997) and criteria for matching bands are described elsewhere (Coyer et al. 1994). All bands between 4 and 23 kb in size were ranked by size and band presence/absence noted for 36 individuals (18 high-flow, 18 low-flow). Genetic structure within and among the low-flow and high-flow populations was tested by analyzing the data matrix as RFLP data (haplotypic) using analysis of molecular variance (AMOVA, Excoffier et al. 1992) in the Arlequin software package (Schneider et al. 1997). As the variance contribution in AMOVA is assessed by a permutation approach rather than tested against an analytically derived  $F$  distribution (as in ANOVA), we used 1000 permutations.

**Reciprocal transplant.** To determine if morphology in *Eisenia arborea* is a plastic response to the local flow environment, a reciprocal transplant study was conducted at both Bird Rock and the Intake Pipes. A total of 48 undifferentiated juveniles were collected from low-flow areas: half were transplanted back into low-flow areas (= transplant controls) and half were transplanted into high-flow areas. The reciprocal was carried out on individuals collected from high-flow areas. An array of eight 1 m PVC pipes was bolted to the seafloor in both the high-flow and low-flow areas (at both Bird Rock and the Intake Pipes) and 6 individuals (3 of each morphotype) were attached with rubber tubing to each pipe, each 16 cm apart.

Controls for manipulation effects, in addition to the transplant controls, consisted of 20 resident tagged individuals that were monitored for growth in the same way as were the transplants. After 3 mo, blade width and bump height were measured with calipers in all treatments to the nearest 0.1 mm. Due to mortality of individuals at both sites (both transplant and controls), the final sample sizes of each high-flow treatment were: Bird Rock = 6 flat residents, 9 flat transplant controls, and 10 bullate transplants; Intake Pipes = 7 flat residents, 14 flat transplant controls, and 10 bullate transplants. Each low-flow treatment consisted of: Bird Rock = 8 bullate residents, 5 bullate transplant controls, and 5 flat transplants; Intake Pipes = 5 bullate residents, 9 bullate transplant controls, and 9 flat transplants. Analysis employed a 3-way ANOVA with site (Bird Rock and Intake Pipes), source (bullate or flat adults in collection area), and flow (high and low) as factors. A  $t$ -test was used to compare transplant controls with unmanipulated (resident) individuals.

**Common garden.** Five juveniles from high- and low-flow environments were transplanted from Santa Catalina Island into a large (1522910 l), low-flow,

high-nutrient tank (the 'Kelp Forest Exhibit') at the Monterey Bay Aquarium (MBA) in Monterey, California, in September 1997. Individuals were transported in chilled seawater and maintained in outdoor tanks with running seawater at the Hopkins Marine Station for several days prior to placement in the MBA. Plastic tubing was attached to rocks in the aquarium with marine epoxy (Z-Spar A788 Splash Zone Compound, Kop-Coat) and the *Eisenia arborea* stipe tied to the tubing so the holdfast was pressed to the substratum. Individuals were monitored as described for the field experiment and analyzed using a *t*-test (treatment = high- or low-flow morphs in collection area) at a significance level of 0.05. The common garden experiment provided an additional perspective on morphological plasticity in response to water motion; if the individuals responded to water motion, both morphotypes would be expected to converge in morphology. In this case, the low-flow morphology is the most likely form, as flows in the aquarium are less than  $2 \text{ cm s}^{-1}$  (R. Phillips, Monterey Bay Aquarium, pers. comm.).

**Reproductive state.** In addition to blade characteristics, the reproductive status of individuals was noted throughout the course of field studies at Santa Catalina Island (summer 1998 to summer 2001). In June 1998, 12 permanent  $10 \times 1 \text{ m}$  transects were marked at each site (Bird Rock and Intake Pipes), 6 in high flow and 6 in low flow (for a total of twenty-four  $10 \text{ m}$  transects) with large railroad spikes and small floats. The presence or absence of 5 or more individuals containing ripe reproductive sori (sorus patches that were visibly sloughing off cells) was noted for each transect every season (spring, summer, fall and winter) from 1998 to 2001. To quantify the percentage of reproductive individuals present in the population at any given time, the density of adults (individuals with  $>10$  blades per crown) per area (no. of individuals  $\text{m}^{-2}$ ) containing 3 or more ripe sori per blade was measured along each transect in April 2001. Data from all transects were collected on the same day. As there were no reproductive individuals at the Bird Rock site for either the high-flow or low-flow populations in April 2001, only the data for the Intake Pipes are shown here.

Table 1. *Eisenia arborea*. Adult morphometrics. All morphological characteristics measured were significantly different between high-flow (High) and low-flow (Low) areas at the protected sites. The ANOVA tables for each comparison are listed under ANOVA results. Bump heights as a function of both flow (high or low) and site (Catalina or San Clemente) were significant, but the effect of flow on blade morphology (low flow = bullate blades) was identical between sites (2-way ANOVA results). Significant results are in **bold**

Blade averages (SD)			ANOVA results					
	High	Low		SS	df	MS	F	p
<b>Santa Catalina Island</b>								
Length (cm)	69.5(14.6)	62.3(9.5)	Effect	1764	1	1764	12	<b>0.0008</b>
			Error	20169	134	151		
Width (cm)	4.9(1.0)	20.0(3.6)	Effect	7763	1	7763	1088	<b><math>3.6 \times 10^{-66}</math></b>
			Error	956.4	134	7		
Thickness (mm)	0.6(0.07)	0.3(0.04)	Effect	1.9	1	1.9	605.8	<b><math>1.5 \times 10^{-51}</math></b>
			Error	0.4	134	0.003		
Bump height (mm)	0.8(0.3)	2.5(1.1)	Effect	93.8	1	93.8	150.3	<b><math>1.2 \times 10^{-23}</math></b>
			Error	83.7	134	0.6		
Holdfast area ( $\text{cm}^2$ )	14.8(16.3)	5.4(2.9)	Effect	1000.3	1	1000.3	6.76	<b>0.01</b>
			Error	6510.1	44	147.9		
<b>San Clemente Island</b>								
Length (cm)	78.1(18.9)	64.9(8.4)	Effect	1343.8	1	1343.8	5.5	<b>0.0253</b>
			Error	7268.2	30	242.3		
Width (cm)	6.3(1.1)	21.7(4.3)	Effect	1840.4	1	1840.4	224.4	<b><math>1.8 \times 10^{-15}</math></b>
			Error	245.9	30	8.2		
Thickness (mm)	0.4(0.05)	0.3(0.06)	Effect	0.1	1	0.1	32.2	<b><math>3.4 \times 10^{-6}</math></b>
			Error	0.1	30	0.003		
Bump height (mm)	1.3(0.4)	3.2(1.0)	Effect	27.6	1	27.6	54.2	<b><math>3.4 \times 10^{-8}</math></b>
			Error	15.3	30	0.5		
<b>2-way ANOVA results</b>								
(Bump heights)	df effect, error		MS effect	MS error	F		p	
Flow	1, 150		33.5	0.65	51.8		<b><math>2.7 \times 10^{-11}</math></b>	
Site	1, 150		7.7	0.65	11.9		<b>0.0007</b>	
Flow $\times$ Site	1, 150		0.05	0.65	0.07		0.7852	

## RESULTS

### Morphometrics

The 2 populations were morphologically distinct in all characters measured on both islands (Table 1). Blade width and bump height were significantly greater in the low-flow populations, while blade length and thickness were greater in the high-flow individuals. Holdfast areas of high-flow juveniles were more than twice the area of those in low-flow areas.

The bullate blades had  $Re^*$  values 3- to 4-fold greater than those of the flat blades. In a flow of 10  $\text{cm s}^{-1}$  (the average high-flow value), the bullate blades  $Re^* = 21$ , whereas the flat blades  $Re^* = 7$ . In a flow of 2  $\text{cm s}^{-1}$  (the average low flow value), the bullate blades  $Re^* = 4$ , whereas the flat blades  $Re^* = 1$ . These values are at the transition between smooth and turbulent boundary layers, with the flat blades tending to a smooth and the bullate blades tending towards a fully rough boundary layer. They do suggest, however, that bullate blades can generate turbulence at low velocities and may have 4 times the nutrient transport rate in low flow areas than the flat blades.

At Bird Rock, where water flow is inversely correlated with depth (increased depth = decreased flow), both blade width and bump height decreased exponentially with increasing water flow and showed 2 major groupings: flat blades with blade widths and bump heights of 6 to 8 cm and 0.5 to 1 mm, respectively, and bullate blades with blade widths and bump heights of 12 to 18 cm and 3.5 to 4.3 mm, respectively (Fig. 4A). The holdfast morphology showed a similar trend as well, with a higher proportion of creeping holdfasts in low flow (>75%) and a similar high proportion of dense holdfasts in high water flow (>75%) (Fig. 4B). Interestingly, the turning point occurred between 2 and 3  $\text{cm s}^{-1}$  (10 to 15 m depth), just above the average velocity measured in low-flow areas.

The effect of water flow on blade morphology at San Clemente Island was identical to that seen at Santa Catalina Island. At the protected site, bump heights and blade widths were significantly greater in low-flow than in high-flow areas and were actually greater than those observed at Santa Catalina (Table 1). At the exposed site on the windward side of San Clemente Island (where there is no protection afforded by depth) however, depth had no effect on blade morphology and all blades could not be distinguished from the high-flow morph at Santa Catalina Island ( $t_9 = 1.8$ ,  $p = 0.33$ ). The absence of the low-flow morphology at the exposed site suggests that low water motion less than 2  $\text{cm s}^{-1}$  (as measured in the low-flow areas at Santa Catalina Island) is required for the morphological variation observed in *Eisenia arborea*. No individuals exhibiting a

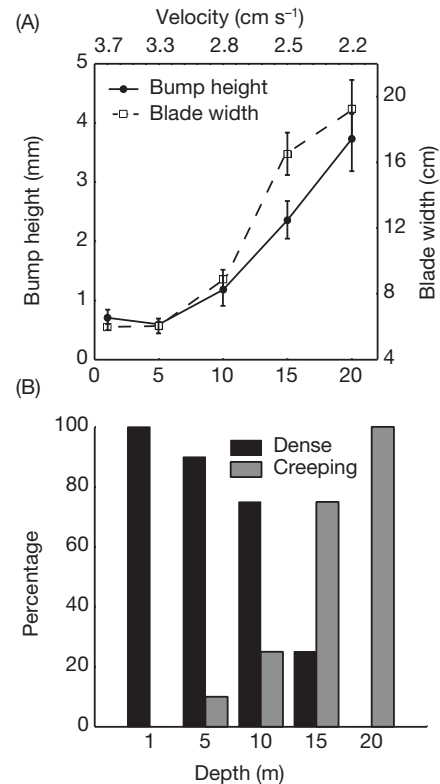


Fig. 4. *Eisenia arborea*. (A) Morphology as a function of velocity. Blade bump height and width increase significantly with decreasing velocity (Log Bump height:  $y = 4.2 - 3.7x$ ,  $R^2 = 0.87$ ,  $p = 0.022$ ; Log Width:  $y = 5.1 - 2.6x$ ,  $R^2 = 0.89$ ,  $p = 0.015$ ). Non-transformed data shown for clarity. Error bars =  $\pm 1$  SE. (B) Holdfast morphology as a function of velocity. High flow morph (depth < 5 m) is dense and compact; low flow morph (> 15 m) is spindly and creeping. Sample size ( $n$ ) = 20 for each depth

low-flow morphology have been observed at exposed sites on Santa Catalina Island, San Clemente Island, or on the mainland in areas where the high-flow morph is present, even at depths exceeding 20 m (J. Engle, UCSB, pers. comm.; L.M.R. pers. obs.).

### Physical environment

Flow was predominantly wave-driven and oscillated at a period of 10 s (data not shown). This period falls well within the range of wave periods normally experienced at this site (NOAA buoy data 1982 to 1993). Water flow at the high-flow site more than doubled during the winter season, but the low-flow site showed very little change (Table 2). Velocities at the exposed SW site on Santa Catalina Island were similar to winter velocities at the high-flow protected site. Maximum velocities (Max), however, were 3-fold greater at the exposed site than those seen at the high-flow protected sites, and more than 6-fold greater than the low-flow

Table 2. Average root mean square (RMS) and maximum (Max) water velocities (SD) at field sites. Flow rates were significantly different between high- and low-flow sites during both the summer and winter months (see ANOVA tables below). The power spectrum of the traces indicate the oscillations have a periodicity of 5 and 10 s, corresponding to the average and dominant wave periods measured at the Catalina Buoy (data not shown). Maximum velocities at 10 m at the exposed site were 6-fold greater than at the 10 m protected site. Significant results are in **bold**

Water velocity (cm s <sup>-1</sup> )	Summer	Winter	Max (SD)		
High flow (5 m)	4.8 (1.3)	9.1 (1.6)	27 (6)		
Low flow (10 m)	1.9 (0.3)	2.9 (0.3)	9 (1.5)		
Exposed 5 m	10.6 (1.4)	N/A	62.7 (14.7)		
Exposed 10 m	7.2 (1.9)	N/A	62.2 (21.7)		
<b>ANOVA results</b>					
Source of variation	SS	df	MS	F	p
Average summer RMS velocities at 'High' and 'Low' flow sites					
Between groups	71.8	1	71.8	74.9	<b>4.1 × 10<sup>-10</sup></b>
Within groups	32.6	34	0.96		
Total	104.4	35			
Average winter RMS velocities at 'High' and 'Low' flow sites					
Between groups	93.0	1	93.0	91.3	<b>1.2 × 10<sup>-5</sup></b>
Within groups	8.2	8	1.0		
Total	101.1	9			

sites, even at greater depths (10 m) where flows are predicted to be lower.

Temperatures were similar at all sites throughout the year (Fig. 5A). The greater variation in temperatures during the summer months was due to tidal oscillation of the thermocline that develops in that season (Cullen et al. 1983, Zimmerman & Kremer 1984), especially during the strong spring tides. During these extreme tidal exchanges, water temperatures changed as much as 6°C in less than 30 min (data not shown). Spectral analysis of the temperature data reveals the tidal nature of these fluctuations, where the dominant frequency is 2 cycles per 24 h period (0.023 mHz). These temperatures are similar to the historical range of temperatures normally experienced by these populations (Fig. 5A, 1982 to 1993 avg) and showed very little variation between years.

Calculated nitrate levels varied between almost zero and 8 µM at all sites, with a peak during the winter months (Fig. 5B). The average predicted nitrate concentration during the warm months (June to October) was 1.15 µM and was not significantly different be-

tween sites or flow treatments (Table 3). The predicted values fall within the range of actual nitrate concentrations measured at the Intake Pipes, Catalina Island (Zimmerman & Kramer 1984).

The light extinction coefficient ( $K_d$ ) for both sunny and cloudy days was 0.13 (SD = 0.02 and 0.004, respectively), comparable to data from clear, oceanic waters (upwelling and turbid areas have  $K_d$  in the range of 0.4 to 2.0; Kirk 1983). Between the high-flow (0 to 10 m depth) and low-flow (15 to 30 m depth) areas at Bird Rock and respectively), summertime PAR varied from 1500 to 500 and 500 to 200 µmol quanta m<sup>-2</sup> s<sup>-1</sup>, respectively. During cloudy conditions at the same location, light levels dropped dramatically to 150 to 40 µmol quanta m<sup>-2</sup> s<sup>-1</sup> in the high-flow areas and 40 to 15 µmol quanta m<sup>-2</sup> s<sup>-1</sup> in low flow. These differences between the high- and low-flow sites could be substantial, but as even at 20 m under cloudy conditions, light levels were sufficiently high (40 µmol quanta m<sup>-2</sup> s<sup>-1</sup>) to saturate photosynthesis in most kelp species (20 to 50 µmol quanta m<sup>-2</sup> s<sup>-1</sup>), including *Eisenia arborea* (Jackson 1977, Gerard 1982, Kopczak 1994, L.M.R. unpubl.

Table 3. Two-way ANOVA results for calculated nitrate concentrations at Santa Catalina Island (June to October) 1998 and 1999. Site = Bird Rock and Intake Pipes; Flow = high and low. No significant differences were observed with site, local water flow, or the interaction between site and flow. Average (SD) nitrate concentrations (µM NO<sub>3</sub><sup>-</sup>) at the Intake Pipes = 1.13 (0.17) and 1.11 (0.11) for high and low flow, respectively; at Bird Rock = 1.19 (0.07) and 1.19 (0.09) for high and low flow, respectively

Source of variation	df Effect, Error	MS Effect	MS Error	F	p
Site	1,30	0.0320	0.016	1.97	0.1702
Flow	1,30	0.0016	0.016	0.09	0.7576
Site × Flow	1,30	0.0004	0.016	0.02	0.8761



data). Differences in light levels, therefore, would not affect photosynthetic rates during the critical summer months (i.e. when most of the photosynthesis takes place) and thus play no role in blade differentiation.

**Genetic analysis**

Significant genetic differences were observed between individuals from high- and low-flow environments (Table 4). A total of 90 unique bands from 4 to 23 kb in size were resolved with the M13 repeat probe, ranging from 9 to 23 bands (mean = 17.2, SD = 3.8) and 11 to 26 bands (mean = 17.0, SD = 3.4) for the 18 individuals in the high- and low-flow populations, respectively. When considering only those bands occurring in at least 5 of the 36 individuals examined, 5 bands occurred primarily or exclusively in high-flow individuals (band numbers: 14, 36, 66, 79, 87; 71 to 100%), whereas 4 bands were most prevalent in low-flow individuals (band numbers: 33, 38, 64, 78; 71 to 75%) (data not shown).

Multilocus M13 DNA fingerprinting revealed highly significant differences between the high- and low-flow populations of *Eisenia arborea*, with nearly 9% of the total genetic variance due to between-population differences (1000 permutations,  $p < 0.001$ ). When the 9 bands that were most prevalent (>70% occurrence) in either population were removed from the AMOVA, the between-population component of total genetic variance was 10-fold less (0.83%) and non-significant ( $p = 0.175$ ) (data not shown). Thus, about 10% of the bands accounted for the observed genetic differentiation.

**Reciprocal transplant**

Blade morphology of transplanted individuals retained characteristics of non-transplanted controls

regardless of flow environment. For both bump height (Table 5) and blade width (Table 6) over the approximately 120 d period of the experiment, the source of transplants (whether adults in the collection area were

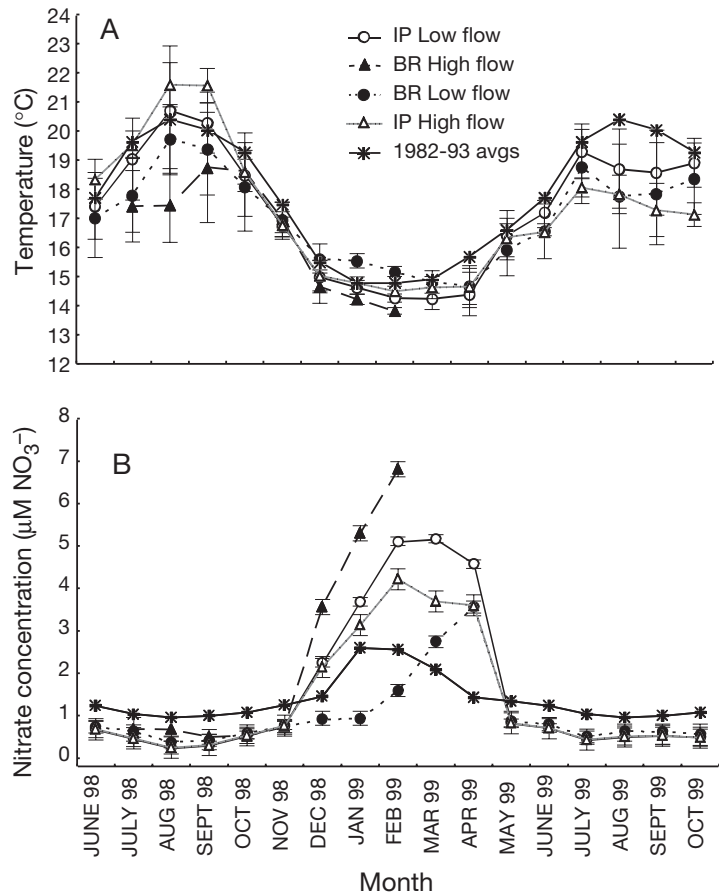


Fig. 5. (A) Monthly average seawater temperatures at Santa Catalina Island, June 1998 to October 1999. BR = Bird Rock (closed symbols), IP = Intake Pipes (open symbols). Circles represent low-flow sites; triangles represent high-flow sites. Monthly temperatures averaged from 1982 to 1993 at the Catalina Buoy (1982-93 avgs) are shown here for reference. No temperature data were collected at BR High flow after January 1999 due to technical difficulties. Error bars =  $\pm 1$  SD. (B) Monthly average nitrate concentrations (calculated) at Santa Catalina Island, June 1998 to October 1999

Table 4. AMOVA analysis of band presence/absence between low- and high-flow populations of *Eisenia arborea*. M13 DNA fingerprinting characterized 90 bands from 18 individuals from each population. The data matrix was analyzed as RFLP data (haplotypic) with the Arlequin software package. Significance of the ratio in variance contribution was assessed with 1000 permutations of the entire genotype between populations. Significant results are in **bold**

Source of variation	df	SS	Variance	% of Variation	p
Among populations	1	27.1	0.95	8.7	<b>&lt;0.0001</b>
Within populations	34	338.7	9.96	91.3	
Total	35	365.9			

Table 5. Averages and ANOVA results for *Eisenia arborea* transplant blade morphometrics. I. Bump height. Site treatment = Bird Rock (BR) and Intake Pipes (IP); Source = bullate adults in collection area (B) and flat adults in collection area (F); Flow = high flow (H) and low flow (L); Controls = back transplant (Back); and unmanipulated resident individuals (Unman). Bump height averages (SD) in mm for Site = BR: 1.6 (0.4) and IP: 2.2 (0.4); Source = B: 2.7 (0.6) and F: 1.3 (0.3); and Flow = H: 2.0 (0.4) and L: 2.1 (0.4). Averages for back transplant controls were not significantly different from unmanipulated controls (*t*-test, BR high flow:  $t = 0.32$ ,  $p = 0.38$ ; BR low flow:  $t = 0.72$ ,  $p = 0.25$ ; IP high flow:  $t = 0.88$ ,  $p = 0.20$ ; IP low flow:  $t = 1.44$ ,  $p = 0.09$ ). Significant results are in **bold**

Averages (SD) of controls (in mm)		Bird Rock		Intake Pipes		
Flow		Back	Unman	Back	Unman	
High		1.52 (0.57)	1.64 (0.70)	1.23 (0.05)	1.09 (0.01)	
Low		2.1 (1.61)	2.78 (1.70)	2.78 (1.68)	1.74 (0.22)	
ANOVA results		SS	df	MS	F	p
Source of variation						
Site	Effect	4.13	1	4.13	3.29	0.0750
Source	Effect	19.83	1	19.83	15.82	<b>0.0002</b>
Flow	Effect	0.0047	1	0.0047	0.0038	0.9514
Site × Source	Effect	2.03	1	2.03	1.62	0.2077
Source × Flow	Effect	0.0019	1	0.0019	0.0015	0.9688
Site × Flow	Effect	0.6983	1	0.6983	0.5570	0.4583
Site × Source × Flow	Effect	0.8441	1	0.8441	0.6734	0.4151
	Error	76.47	61	1.25		

bullate or flat) accounted for the majority of the variation observed between the transplants. The manipulation of transplants had no significant effect on blade morphology (Tables 5 & 6).

### Common garden

As in the field transplant experiment, both morphotypes produced blades consistent with their source

environment and were minimally affected by local water flow. Only 3 individuals of each morphotype survived repeated attacks by a territorial fish, but all were recognizable as either high-flow or low-flow despite growing under identical conditions and immediately adjacent to one another over a 4 yr period. Both blade width and bump height were significantly greater in the low-flow morphotype than in the high-flow form (width:  $t_{27} = 116.98$ ,  $p < 0.0001$ ; bump height:  $t_{27} = 50.92$ ,  $p < 0.0001$ ). The average (SE) blade width and

Table 6. Averages and ANOVA results for *Eisenia arborea* transplant blade morphometrics. II. Blade width. See Table 5 for explanation of symbols used. Blade width averages (SD) in cm for Site = BR: 11.4 (1.6) and IP: 10.8 (1.6); Source = B: 14.1 (1.0) and F: 8.1 (0.8); and Flow = H: 11.6 (1.7) and L: 10.5 (3.1). Averages for back transplant controls were not significantly different from unmanipulated controls (*t*-test, BR high flow:  $t = 0.57$ ,  $p = 0.29$ ; BR low flow:  $t = 1.56$ ,  $p = 0.09$ ; IP high flow:  $t = 0.88$ ,  $p = 0.20$ ; IP low flow:  $t = 1.94$ ,  $p = 0.05$ ). Significant results are in **bold**

Averages (SE) of controls (in cm)		Bird Rock		Intake Pipes		
Flow		Back	Unman	Back	Unman	
High		8.8 (1.6)	10.0 (3.2)	6.8 (0.77)	6.8 (1.3)	
Low		16.5 (2.0)	12.0 (4.3)	13.4 (5.1)	23.0 (6.5)	
ANOVA results		SS	df	MS	F	p
Source of variation						
Site	Effect	5.50	1	5.50	0.29	0.59
Source	Effect	549.50	1	549.50	28.87	<b><math>1.28 \times 10^{-6}</math></b>
Flow	Effect	17.76	1	1776.0	0.93	0.34
Site × Source	Effect	7.16	1	7.16	0.38	0.54
Source × Flow	Effect	8.09	1	8.09	0.42	0.52
Site × Flow	Effect	25.90	1	25.90	1.36	0.25
Site × Source × Flow	Effect	53.21	1	53.21	2.80	0.10
	Error	1161.0	61	19.03		

bump height of the low-flow morphotype was 25.4 cm (1.2) and 4.5 mm (1.2), respectively. In the high-flow morphotype, however, blade width was only 8.7 cm (0.8) and bump height was 0.9 mm (0.8). The dimensions are in the same range as adults from low- and high-flow areas in the field (see Table 1), but it is important to note they were not identical (as in the reciprocal transplant experiment). For example, the average width of high-flow individuals in the MBA was 9 cm (0.8), whereas in the field the average width was 5 cm (1). Also, flat individuals in the MBA appeared in poor physical conditions (few blades, heavy epiphyte load, rapid blade disintegration) whereas the low-flow morph appeared robust and highly productive (very large, clean blades, many new recruits).

### Reproductive state

The percentage of reproductive individuals in the high- and low-flow populations from 1998 to 2001 at the Intake Pipes was greater than or equal to 50% of the total adult population. On average in low-flow, 50% (SD = 0.4) were reproductive, while in the high-flow areas 88% (SD = 0.1) were reproductive, indicating that spores are being produced in both populations simultaneously.

### DISCUSSION

Many species of algae are thought to produce different morphologies in response to local water flow or wave exposure (e.g. *Laminaria longicuris*, Gerard & Mann 1979; *L. japonica*, Kawamata 2001; *Macrocystis integrifolia*, Druehl & Kemp 1982; *Nereocystis luetkeana*, Koehl & Alberte 1988; *Durvillea potatorum*, Cheshire & Hallam 1989; *Sargassum polyceratum*, De Ruyter van Steveninck & Breeman 1987; *S. muticum*, Andrew & Viejo 1998; *Fucus vesiculosus*, Bäck 1993, Kalvas & Kautsky 1993, Ruuskanen & Bäck 1999; *Fucus* spp., Munder & Kremer 1997; *F. spiralis*, Anderson & Scott 1998; *Pelagophycus porra*, Miller et al. 2000; *Egregia menziesii*, Blanchette et al. 2002). These studies, however, have not addressed the relative contribution of both genetic and environmental factors simultaneously, information that is critical for understanding the evolutionary significance of the morphological variability observed. For the kelp *Eisenia arborea* in southern California, we have shown that only water motion varies with changes in morphology (i.e. light, temperature, and nutrients do not affect morphology directly), that morphological variation exists along a gradient in water motion, that low water motion is a requirement for morphological variability,

and perhaps most importantly, that the variation in morphology is accompanied by genetic variation, indicating strong selection against the 'wrong' phenotype over small spatial scales. Indeed, the 2 morphs exhibit potentially adaptive characteristics: the low-flow morph produces large bumps that increase turbulence at velocities experienced in the field, thinner and wider blades to decrease diffusional distances and maximize surface area for nutrient uptake, and smaller holdfasts to minimize total energy requirements. The higher level of turbulence produced by bumps could enhance mass transport to bullate blades in low-flow areas ( $2 \text{ cm s}^{-1}$  or less) by a factor of 4, relative to flat blades. In contrast, the high-flow morph produces thick, flat, narrow blades to decrease drag and prevent breakage, plus a large holdfast to decrease the probability of dislodgement. All high-flow morphs in the reciprocal transplant experiment literally dissolved in less than 6 mo in the low-flow environment. In fact, no 'foreign' morphs have been observed in either low-flow or high-flow areas in the field. These observations support the hypothesis that in *E. arborea* there is strong selection on morphology resulting in higher survivorship and fitness for those individuals with the 'appropriate' morphology (i.e. which is best suited to the local flow regime).

In addition to nutrient transport issues, morphology may be a response to mechanical aspects of flow such as breakage or dislodgement. Thick blades and a large, secure holdfast may be energetically costly to individuals in the low-flow environment, where the risk of breakage or dislodgement is low, yet may be critical in high-flow areas, where maximum velocities and the concomitant drag forces can be more than an order of magnitude greater (Kawamata 2001). Kawamata (2001) demonstrated this relationship for *Laminaria japonica* on the coast of Japan at high- and low-flow sites comparable to those at Santa Catalina Island, and found that the sheltered morphology (flows of  $5 \text{ cm s}^{-1}$ ) had significantly higher drag and lower survivorship in high flow ( $11 \text{ cm s}^{-1}$ ) than the exposed morphology of *L. japonica*. Thus, the morphological variation exhibited by *Eisenia arborea* in the 2 flow environments may represent adaptations to enhance fitness under different nutrient availability and drag force regimes with significant ecological and evolutionary consequences. The recurrence of the link between morphology and water motion in *E. arborea* on 2 separate islands indicates this may be an important phenomenon in not only this species, but in other kelps and non-motile organisms as well.

Can the divergence of the high- and low-flow populations be explained by limited gene flow (maintained by limited gamete dispersal or by incompatible gametes of the 2 morphotypes; Endler 1977, Gottlieb

1984, Barton & Hewitt 1989, Primack & Kang 1989)? Kelp spores are capable of dispersing many kilometers from the point of release (Reed et al. 1988, 1992, Gaylord et al. 2002), but little is known about the dispersal capabilities of *Eisenia arborea*. *E. arborea* spores, however, are similar in size and composition to the far-dispersing *Pterygophora californica* spores (Reed et al. 1999) and would therefore be expected to have a similar high dispersal potential. Widespread dispersal of *E. arborea* is implied by the lack of sequence variation in the rDNA ITS1 region of individuals separated by >400 km (data not shown). Furthermore, newly settled *E. arborea* resulting from the common garden experiment in the Monterey Bay Aquarium (e.g. offspring of the original transplants) were able to disperse 15 m from the parent in a low-flow environment (data not shown). Yet, in the field, *E. arborea* settlement is highly clustered around adults (data not shown), suggesting that the majority of spores recruit over small spatial scales (<1 m; Gaylord & Gaines 2000). Limited gamete dispersal in *E. arborea* may therefore account for at least some of the observed genetic differences between morphotypes but is unlikely to play a major role.

Gamete compatibility between the 2 morphotypes is unknown, but probably is high, as intergeneric and even interfamilial crosses of kelp species in the laboratory and field routinely produce large (although usually sterile) sporophytes (Sanbonsuga & Neushul 1978, Lewis 1996). Additionally, several putative hybrid individuals exhibiting intermediate morphological characteristics of the high- and low-flow *Eisenia arborea* morphotypes possessed genetic markers characteristic of each and were fully capable of producing viable spores (data not shown). Thus, it is likely that gametes of the 2 morphotypes can produce viable offspring and that mixing between the 2 populations occurs in the field.

A recent study of the elk kelp *Pelagophycus porra* off southern California (USA) and Baja California (Mexico) demonstrated significant genetic differentiation between morphologically distinct leeward and windward populations over a spatial scale of 10 to 360 km (Miller et al. 2000). Although the lack of divergence in nuclear rDNA-ITS and the chloroplast *trnL* intron precluded designation as different species, the genetic differentiation revealed by random amplified polymorphic DNA (RAPD) analysis suggested that the leeward populations comprised a species *in statu nascendi* (Miller et al. 2000). The genetic and morphological differentiation we observed in *Eisenia arborea* is similar to that observed in *P. porra*, lending further support for the importance of water motion in the ecology and evolution of kelps.

Our transplant and genetic data suggest that water motion selects for, and maintains, 2 distinct morpho-

types of *Eisenia arborea*. The potential of the morphotypes to evolve into separate species or the possibility that the morphotypes currently can be considered species *in statu nascendi*, however, awaits further studies. For example, comparison of rDNA ITS1 and ITS2 sequences between the 2 morphotypes produced equivocal results (data not shown), even though the fast-evolving rDNA ITS regions often have been used to distinguish closely related species and subspecies of marine algae (Goff et al. 1994, van Oppen et al. 1994, 1995, Peters et al. 1997, Serrão et al. 1999). Sequence evaluation of other genes and/or introns from more individuals of *E. arborea* in each flow environment, as well as the use of genetic markers sensitive to variable degrees of gene flow (e.g. microsatellites), are necessary before the taxonomic status of *E. arborea* morphotypes can be fully understood. In addition, it is not known whether the predicted differences in nutrient transport (based on  $Re^*$ ) would result in differences in growth rate or fitness between the 2 morphs. Small-scale water velocity measurements at the blade surface are required for more accurate estimation and comparison of turbulent transport to bullate and flat blades. In conjunction with small-scale velocity data, knowledge of the relative performance of each morph (i.e. photosynthetic rate, growth, or survivorship) under high- and low-flow conditions is necessary to understand fully the mechanism of selection acting on morphology in *E. arborea*.

Kelps may be an excellent model system for understanding the environmental and genetic components of local adaptation and speciation. Many species are ecologically and economically important. Most species can be easily transplanted for various manipulative experiments, tagged and monitored for demographic studies, and subjected to population genetic analysis using a wide variety of techniques. The coupling of these approaches in kelps should provide a wealth of information on adaptation and speciation in algae as well as marine organisms in general.

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