

Evolutionary History and Adaptive Significance of Respiratory Structures on the Legs of Intertidal Porcelain Crabs, Genus *Petrolisthes*

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ABSTRACT

Semiterrestrial and terrestrial crabs have evolved multiple strategies for aerial respiration. An uncommon strategy for aerial respiration is seen in porcelain crabs, genus *Petrolisthes*, where decalcified areas on the meral segments of the walking legs are used as respiratory structures. Here, the evolutionary history and adaptive significance of these structures in porcelain crabs is examined. Interspecific variation in leg membrane size is from 0% to 60% of the surface area of the meral segment. Leg membrane relative size is positively correlated with body size across species but not within one species, *Petrolisthes cinctipes*. Phylogenetic analyses suggest that leg membranes are ancestral to one of two eastern Pacific *Petrolisthes* clades. Comparative analyses using phylogenetic independent contrasts indicate a relationship between leg membrane relative size and body size that is phylogenetically independent. In large-bodied intertidal species, whole-animal lactate accumulation during aerial incubation is 200%–300% higher when the leg membranes are obscured, indicating that the leg membranes are functional respiratory structures in these species. Thus, it is possible that leg membranes have facilitated the evolution of larger body sizes by providing additional respiratory surfaces to accommodate the associated higher metabolic demands.

Introduction

Intertidal organisms are routinely exposed to a suite of rapidly fluctuating physical factors as a result of the low-tide-period

shift from a marine to a terrestrial habitat. Organisms that live in the middle and upper intertidal zones can spend over 50% of their lives out of water and exposed to terrestrial conditions (Stillman and Somero 1996). Aerial exposure creates a great physiological challenge for most aquatically respiring organisms, as they generally have respiratory structures that are well suited for gas exchange while immersed but not when emersed (Newell 1979). Most aquatic organisms use gills as the main site for gas exchange, and while gills function very well in water, they do not perform well when in air. The drop in gas exchange performance is primarily due to problems involved with preventing the collapse of the gills' fine lamellar structures while emersed (Copeland 1968). These problems greatly affect the respiratory structures of intertidal and terrestrial crabs, which are some of the most successful (in terms of diversity) of marine or aquatic organisms living in terrestrial environments.

Crabs have evolved many strategies for maintaining an aerobic poise while out of water (DeFur 1988). Some crabs retain or recirculate branchial water and are thus able to keep their gills in an "aquatic" and oxygenated environment during periods of emersion (DeFur et al. 1983; Depledge 1984). However, in most cases of terrestrial and semiterrestrial crabs, the branchial chamber is not filled with water during periods of emersion. In these cases, gills tend to be greatly reduced (in both number and surface area; Gray 1957; Hawkins and Jones 1982) although the remaining gills are structurally reinforced by thicker epithelial and chitin layers to prevent collapse while emersed (Copeland 1968). In some cases, gills are replaced with a functional lung, created by enlarging the carapace and adding increased vascularization to the inner lining of the branchiostegites (Farrelly and Greenaway 1994).

Recently, an additional morphological character facilitating aerial gas exchange in intertidal crabs has been described. This character is a decalcified area on the meral segment of the walking legs and has been described in two distantly related groups of crabs: brachyuran Ocypodid ghost crabs in the genera *Scopimera* and *Dotilla* from Australia (Maitland 1986), and anomuran porcelain crabs in the genus *Petrolisthes* (Jensen and Armstrong 1991; Stillman and Somero 1996). In both groups, these decalcified areas (termed "leg membranes" in Stillman and Somero 1996) serve as aerial respiratory structures during periods of increased metabolic demands, allowing the crabs to remain aerobic during the emersion period (Stillman and Somero 1996).

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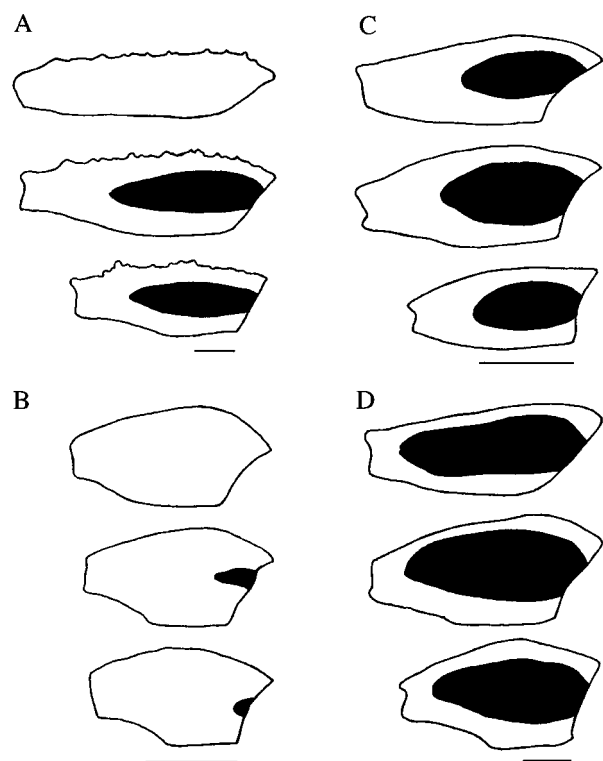


Figure 1. Leg membrane phenotype variation. Tracings of photographs of the ventral side of the meral segment of walking legs from four species, showing occurrence of leg membranes (areas shaded by black). Legs are arranged leg 1 to leg 3, top to bottom, for each species. Species are (A) *Petrolisthes tiburonensis*, (B) *Petrolisthes granulatus*, (C) *Petrolisthes cabrilloi*, and (D) *Petrolisthes tuberculosus*. Scale bars represent 4 mm.

A preliminary survey of the genus *Petrolisthes* (Stillman and Somero 1996) indicated that the leg membranes are present in 16 of 79 species examined and that the interspecific variation in leg membrane relative size is considerable. The genus *Petrolisthes* is relatively large, with more than 100 species worldwide (Stillman 1998). Because of the large number of species within this genus, as well as the large diversity in microhabitat conditions as created by both biogeographic and local distribution patterns, these crabs provide an excellent study system for addressing the adaptive significance of the presence of leg membranes and the variation in leg membrane size using the comparative method (Harvey and Pagel 1991; Garland and Adolph 1994).

Here, the occurrence, diversity, and functional and evolutionary significance of leg membranes throughout the genus *Petrolisthes* is presented. Both interspecific and intraspecific variation in leg membrane size is analyzed with respect to distribution and body size. Leg membrane function is examined by measuring the respiratory status, as indexed by lactic acid ac-

cumulation, during emersion of crabs with their leg membranes obscured (Stillman and Somero 1996).

The evolutionary origins and adaptive significance of leg membranes are examined using a phylogenetic tree for *Petrolisthes* based on molecular sequence data from the 16s rDNA gene (Stillman 1998). Phylogenetically independent contrasts (Felsenstein 1985) were generated for evolutionary analyses of the relationships between leg membrane relative size, body size, and vertical distribution. Stillman and Somero (1996) showed that, in small specimens, the physiological need for leg membranes as aerial respiratory structures was not high. Maximum body sizes among *Petrolisthes* range from ~4 to >30 mm carapace length (a much greater range than used in intraspecific comparisons in Stillman and Somero 1996), thus questions regarding the proximate and ultimate relationship between leg membrane relative size and body size can be addressed.

Material and Methods

Specimen Collection and Preparation

Specimens used either were from the collections of the Los Angeles County Museum of Natural History and the National Museum of Natural History or were hand collected by the author. Museum specimens showing signs of exoskeleton deterioration were not used. Specimens collected by the author were either dried or were fixed in formalin and stored in 70% ethanol. Before photographic documentation, the carapace length and width of the specimen was measured, and then walking legs were removed and placed under a dissecting microscope equipped with a photographic extension tube. For at least one specimen of each species, legs were dissected along the thin axis of the leg (anterior-posterior), and the muscle tissue was removed (as in Stillman and Somero 1996). This preparation gave the most striking photographic documentation of leg membrane morphometrics, but nondissected preparations were equally sufficient for data collection. Color photographs were made of the ventral surface of each leg, and glossy prints were used in subsequent analyses.

Leg Membrane Relative Size Analysis

To quantify the relative size of the leg membrane, the meral segment and the leg membrane portion of the meral segment were excised from the photograph with a razor blade. Both segments were weighed on an analytical balance to the nearest 0.1 mg. Percentage coverage of the meral surface area by the leg membrane was calculated as the weight of the leg membrane segment divided by the weight of the leg membrane segment plus the non-leg-membrane segment of the meral surface. The leg membrane relative size thus represents the percentage of surface area of the ventral side of the meral segment that is occupied by the leg membrane. This definition of leg membrane

Table 1: Leg membrane relative size variation in the genus *Petrolisthes*

Species ^a	<i>n</i>	Maximum Size ^b	Distribution ^c	Leg 1 ^d	Leg 2 ^d	Leg 3 ^d
<i>Petrolisthes</i> :						
<i>brachycarpus</i>	1	6.0	?	.0 ± n/a	6.3 ± n/a	5.4 ± n/a
<i>cabrilloi</i>	1	15.4	4	28.1 ± n/a	38.2 ± n/a	33.3 ± n/a
<i>cinctipes</i>	88	21.1	4	38.8 ± 3.1	40.5 ± 3.4	31.0 ± 3.6
<i>galapagensis</i>	1	10.1	?	34.3 ± n/a	40.7 ± n/a	39.7 ± n/a
<i>gertrudae</i>	1	4.4	4	18.5 ± n/a	12.8 ± n/a	23.6 ± n/a
<i>gracilis-1</i> ^e	4	10.4	5	.0 ± .0	8.1 ± .7	9.1 ± .8
<i>gracilis-2</i> ^e	4	10.4	5	14.8 ± 2.4	29.9 ± 4.2	33.4 ± 6.0
<i>granulosus</i>	5	14.5	5	.0 ± .0	4.7 ± 1.5	1.1 ± .0
<i>hians</i>	1	5.6	?	2.1 ± n/a	12.5 ± n/a	7.3 ± n/a
<i>hirtipes</i>	16	11.1	3	50.3 ± 5.0	54.5 ± 3.6	51.6 ± 3.9
<i>laevigatus</i>	25	23.8	4	59.3 ± 4.6	60.0 ± 4.5	56.1 ± 6.4
<i>lewisi austrinusus</i>	15	6.0	3	7.0 ± .0	12.5 ± 8.1	5.1 ± 1.3
<i>lewisi lewisi</i>	1	6.0	3	6.9 ± n/a	11.8 ± n/a	5.1 ± n/a
<i>nigrunguiculatus</i>	1	9.6	?	40.0 ± n/a	44.9 ± n/a	47.3 ± n/a
<i>quadratus</i>	1	7.0	6	.0 ± n/a	22.9 ± n/a	14.2 ± n/a
<i>rathbunae</i>	1	18.6	2	44.0 ± n/a	41.7 ± n/a	34.5 ± n/a
<i>schmitti</i>	1	6.0	2	12.5 ± n/a	22.5 ± n/a	25.1 ± n/a
<i>tiburonensis</i>	1	11.8	3	.0 ± n/a	27.0 ± n/a	33.4 ± n/a
<i>tonsorius</i>	1	11.6	?	59.5 ± n/a	56.1 ± n/a	53.2 ± n/a
<i>tridentatus</i> ^f	2	6.0	5	.0 ± .0	18.4 ± 2.8	12.8 ± 3.1
cf. <i>tridentatus</i> ^f	17	6.3	5	19.4 ± n/a	28.2 ± 4.7	29.1 ± 5.2
<i>tuberculatus</i>	21	22.0	2	49.8 ± 5.0	52.8 ± 4.8	50.0 ± 8.4
<i>tuberculosis</i>	10	30.0	1	53.2 ± 4.5	57.1 ± 6.4	55.9 ± 4.5
<i>violaceus</i>	23	27.0	3	50.5 ± 5.4	47.5 ± 3.8	39.0 ± 3.5
<i>Allopetrolisthes spinifrons</i>	1	20.3	2	36.7 ± n/a	36.3 ± n/a	44.6 ± n/a

^a Species listed are all of those examined that possessed leg membranes. Species surveyed include those in the collections of the Los Angeles County Museum of Natural History and the National Museum of Natural History.

^b Maximum body size in millimeters of carapace width. Data are from Haig 1960 and, for *Petrolisthes gertrudae*, from Werding 1996.

^c Maximal vertical distribution in the intertidal zone coded from 1 = subtidal to 6 = splash zone. A question mark indicates that no information on the vertical distribution was found. Data are from Chace and Hobbs 1969; Romero 1982; Weber Urbina 1986; Jensen and Armstrong 1991; Werding 1996; Stillman 1998.

^d Data represent mean percentage of meral surface area occupied by leg membrane ± 1 SD, or n/a where *n* = 1.

^e Distinction between two types of *Petrolisthes gracilis* made only on the basis of leg membrane phenotype.

^f Distinction between two types of *Petrolisthes tridentatus* made by geographic separation and color in life; *P. tridentatus* is the Caribbean form, *P. cf. tridentatus* the Pacific form.

size is the one that is explicitly used in this report; no reference is made to the actual size (e.g., area in mm²) of the leg membrane. The three pairs of walking legs are referred to as leg 1 to leg 3 (anterior to posterior).

Leg Membrane Function

For analysis of leg membrane function, specimens were collected and placed into flow through aquaria at an on-site laboratory, where they were continuously immersed. Specimens were allowed the maximal time available to acclimate to similar conditions. *Petrolisthes granulosus*, *Petrolisthes violaceus*, *Petrolisthes laevigatus*, and *Petrolisthes tuberculatus*

were collected at Las Cruces, Chile (33°33'S, 71°36'W), from a bouldery intertidal zone habitat adjacent to Universidad Católica's marine laboratory, the Estación Costera del Investigaciones Marinas. These four species were collected on October 12, 1997, and used in experiments on October 17–20, 1997. *Petrolisthes hirtipes* were collected August 15, 1997, at Pelican Point, Cholla Bay, Puerto Peñasco, Mexico (31°39'N, 113°15'W) and used in experiments on August 18, 1997. The largest specimens available from each species were used in the experiments.

The functional properties of leg membranes from a number of species were examined following the procedure in Stillman and Somero (1996). Leg membranes were obscured by appli-

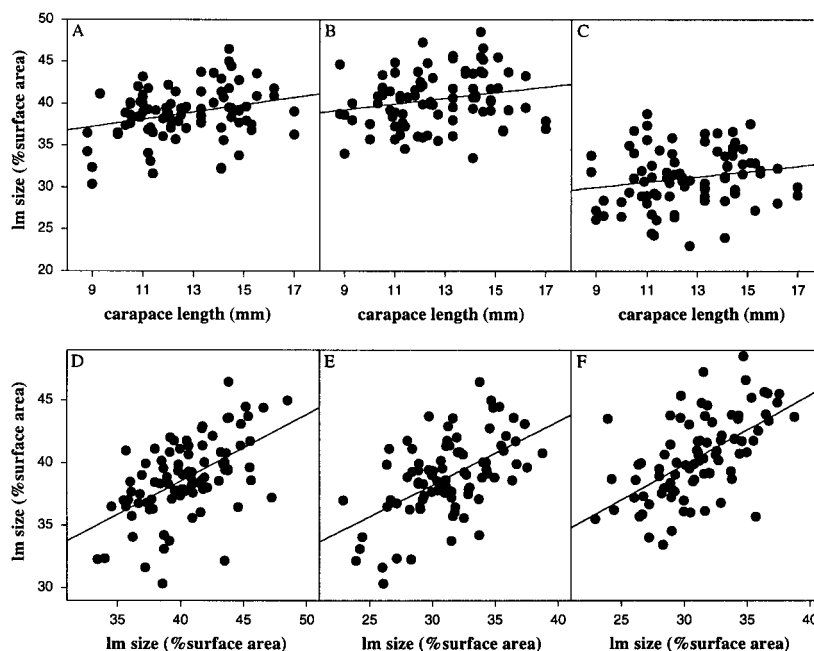


Figure 2. Variation of leg membrane relative size in *Petrolisthes cinctipes*. Leg membrane relative size as a function of body size on (A) leg 1, (B) leg 2, and (C) leg 3. Leg membrane relative size on one leg as a function of leg membrane relative size on another leg for comparisons of (D) leg 1 (Y-axis) versus leg 2 (X-axis), (E) leg 1 (Y-axis) versus leg 3 (X-axis), and (F) leg 2 (Y-axis) versus leg 3 (X-axis). Regression coefficients for each plot are in Table 2.

cation of two thin coats of nail polish (Revlon creme). Only a few legs were painted at a time to minimize the time that the animals spent out of water. Crabs had either all of their legs painted or none of their legs painted (a control for the effect of the painting, where half of the legs were painted, was omitted because no effect was seen in previous studies [Stillman and Somero 1996]). Crab legs were painted 2 d before experimentation began. During the experimental incubation, crabs were incubated in air at elevated body temperatures that were selected to represent warm but not extreme temperatures during low tide (25°–28°C for temperate species and 35°C for tropical species). Following a 5-h incubation period, crabs were frozen immediately by freeze clamping and stored at $\geq -70^{\circ}\text{C}$.

To assay total body lactate, frozen crabs were ground with a mortar and pestle under liquid N_2 and immediately placed into 2 mL of 0.75 M HClO_4 g body mass $^{-1}$. The mixture was incubated on ice for 10 min and then centrifuged at 10,000 g for 10 min. The supernatant was neutralized to pH 7.0 by addition of 3 M KOH, incubated on ice for 10 min, and then centrifuged at 10,000 g for 10 min to remove the KClO_4 precipitate. The resulting supernatant was held on ice and used within 1–2 h for enzymatic determination of lactate concentration with a commercial L-lactic acid kit (Boehringer Mannheim), using the method described by Noll (1984).

Phylogenetic Analyses

Molecular sequence data of the 16s rDNA mitochondrial gene (~450 base pairs) were obtained by PCR amplification, using the universal primers 16SAR and 16SBR (Palumbi et al. 1991) and genomic extracts of frozen tissue and cycle sequencing methods as described in detail elsewhere (Stillman 1998). The phylogenetic trees used here were generated from aligned sequences using neighbor joining of bootstrapped distance and parsimony matrices generated in the PHYLIP software package (Felsenstein 1989), as described elsewhere (Stillman 1998). Independent contrasts (Felsenstein 1985) were generated using the CAIC software package (Purvis and Rambaut 1995) and the results of PHYLIP analyses. Contrasts of leg membrane relative size, body size (mm carapace length), and vertical intertidal position were generated. These contrasts were used in linear regression analyses where the regression was forced through the origin, as is required for analyses of independent contrasts (Purvis and Rambaut 1995).

Results

Occurrence and Size Variation of Leg Membranes

A total of 101 species that are currently, or were formerly, classified within the genus *Petrolisthes* (including 95 species of

Table 2: Results of regression analyses from Figures 2 and 3

	<i>b</i>	<i>m</i>	<i>r</i> ²	<i>F</i>	<i>P</i>
Figure 2:					
A, leg 1 versus body size	33.33	.43	.077	7.1	.0093
B, leg 2 versus body size	36.20	.34	.045	4.1	.0474
C, leg 3 versus body size	27.15	.31	.034	3.1	.0858
D, leg 1 versus leg 2	17.25	.53	.309	38.5	1.9×10^{-8}
E, leg 1 versus leg 3	23.03	.51	.301	37.1	3.1×10^{-8}
F, leg 2 versus leg 3	23.10	.57	.336	42.1	$.5 \times 10^{-8}$
Figure 3:					
A, Leg 1 versus body size	-4.71	2.19	.504	18.31	.0005
B, Leg 2 versus body size	12.84	1.55	.425	14.76	.0010
C, Leg 3 versus body size	10.68	1.47	.354	9.30	.0073
D, Total versus body size	6.38	1.73	.454	14.98	.0011

Note. Linear regression parameters from $y = b + mx$; *F* and *P* are results of regression ANOVA (Fig. 2A–2F: *df* = 1, 86. Fig. 3: A, *df* = 1, 17; B, *df* = 1, 20; C, *df* = 1, 18; and D, *df* = 1, 18.

Petrolisthes, three species of *Allopetrolisthes*, one species of *Liopetrolisthes*, one species of *Neopetrolisthes*, and one species of *Parapetrolisthes*) were examined for the presence of leg membranes (see Stillman 1998 for species list). Leg membranes were only present in eastern Pacific and western Atlantic species, where they are found on 23 out of a total of 55 species of *Petrolisthes* (20 : 42 eastern Pacific and 3 : 13 western Atlantic) and one out of three *Allopetrolisthes*. The leg membranes vary in size (where present) from 1% to 60% of the meral surface area and are sometimes present on only two of three pairs of walking legs (Fig. 1; Table 1).

For two species, there exists sufficient variation in leg membrane relative size to warrant the report of two different morphotypes: *Petrolisthes gracilis* and *Petrolisthes tridentatus*. In *P. gracilis*, one morphotype has no leg membranes on leg 1 and small leg membranes on the other legs (8%–9% surface area). The other morphotype of *P. gracilis* has leg membranes on all three legs (15%–33% surface area; Table 1). In *P. tridentatus*, Pacific specimens (*P. cf. tridentatus*) have a membrane on all three legs (20%–30% surface area), whereas, in the Atlantic specimens, there are no membranes on the first legs and small membranes on legs 2 and 3 (13%–19% surface area; Table 1).

Intraspecific variation of leg membrane relative size was examined in *Petrolisthes cinctipes*. Leg membranes from 88 individuals were measured and the leg membrane relative sizes were plotted as a function of body size (Fig. 2A–2C). Plots were made comparing leg membrane relative size on one leg to leg membrane relative size on a second leg (Fig. 2D–2F). Regression analyses indicate that, while leg membrane relative size is not correlated with body size, individuals with large leg membranes on one leg tend to have large membranes on the other two legs as well (Table 2). Interindividual genetic or ontogenetic differences explain about 30% ($r^2 = 0.30$ – 0.34) of the variation in leg membrane relative size in *P. cinctipes* (Table 2).

Interspecific variation in leg membrane relative size is positively correlated with each species' maximum body size in all legs and for total leg surface area covered by leg membrane (Fig. 3A–3D; Table 2). Body size explains as much as 50% ($r^2 = 0.35$ – 0.50) of the variation in leg membrane relative size (Table 2).

Leg Membrane Function

The role of leg membranes as aerial respiratory structures was examined by an interspecific comparison of the effects of emersion on lactate accumulation in species distributed in different intertidal zones. Obscuring the leg membranes resulted in large differences in whole-animal lactate accumulation in intertidal species that have large leg membranes (i.e., *P. hirtipes*, *P. laevigatus*, and *P. cinctipes* (Fig. 4; ANOVA, $P < 0.05$). Obscuring the leg membranes, however, did not have an effect in *P. granulatus*, a high intertidal species with very small leg membranes (Figs. 1, 4; Table 1). Obscuring the leg membranes of *P. violaceus*, a low-intertidal, large-bodied crab, and *P. tuberculatus*, a subtidal, extremely active crab, did not result in lactate accumulation levels that were different from control levels (Fig. 4). However, in *P. violaceus* and *P. tuberculatus*, both treatment and control lactate accumulation were high (Fig. 4) unlike *P. granulatus*, where both treatment and control lactate accumulation were low. Lactate accumulation of control specimens was highest in *P. violaceus* and *P. tuberculatus* and lowest in *P. granulatus* and *P. hirtipes*, two small-bodied crabs (Fig. 4; Table 1).

Leg Membrane Evolution

The evolutionary history of the leg membrane phenotype was examined by mapping the character on a phylogenetic tree of

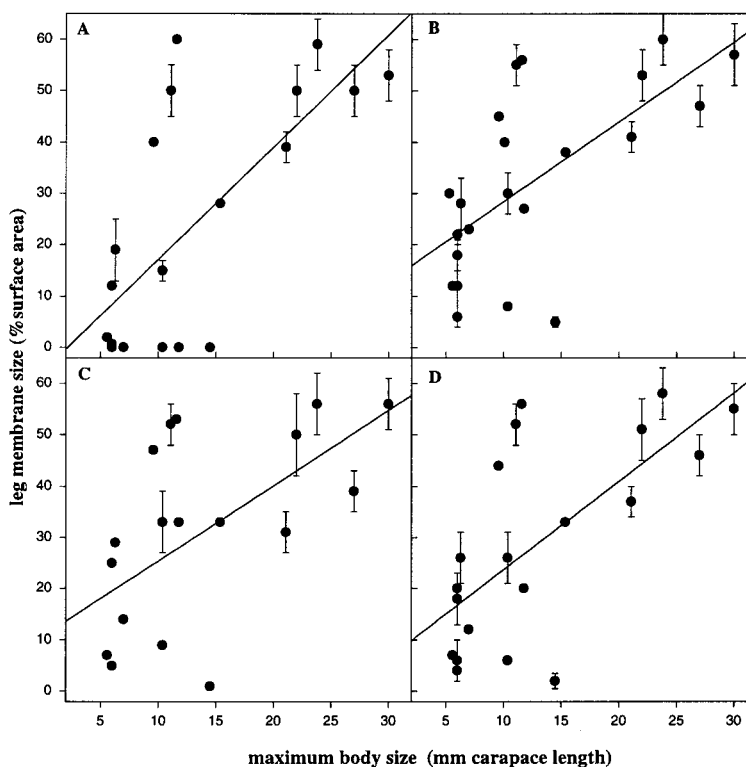


Figure 3. Interspecific comparison of the relationship of leg membrane relative size to body size. Plots are of (A) leg 1, (B) leg 2, (C) leg 3, (D) total leg surface area. Each point represents a different species, and error bars are 1 SD. Data are from Table 1. Regression coefficients for each plot are in Table 2.

the genus (Fig. 5, *thick solid and dashed lines*). The leg membranes are only found in one of two clades and are an ancestral condition in that clade (Fig. 5).

Independent Contrasts Analyses

A phylogenetics-based comparative approach was employed to examine the adaptive significance of the leg membrane phenotype. Independent contrasts, generated from the phylogenetic tree in Figure 6, of the total percentage of meral surface area covered by leg membranes, were plotted against contrasts of maximum body sizes and vertical intertidal zone distributions (Fig. 7). Results of regression analyses indicate that there is a positive correlation between leg membrane relative size contrasts and body size contrasts (Fig. 7, *filled circles*, regression line shown) but not between leg membrane relative size contrasts and vertical position contrasts (Fig. 7, *open circles*, no regression shown). The slope of the regression line is 0.017 (Fig. 7), identical to the slope found by regression analyses of nonphylogenetically corrected data (Fig. 3D; Table 2).

Discussion

Leg Membrane Relative Size Variation

The size of leg membranes varies from 0% to 60% of the surface area of the ventral side of the meral segment of the walking legs (Fig. 3; Table 1). Intraspecific variation in leg membrane relative size is less than the amount of interspecific variation (Figs. 2A–2C, 3A–3D; Table 1). The correlation of leg membrane relative size between legs of individual specimens (Fig. 2D–2F; Table 2) suggests that genetic or ontogenetic effects may account for about 30% of the leg membrane relative size variation (Table 2). To demonstrate that the intraspecific variation in leg membrane relative size is caused by ontogenetic effects, it would be necessary to be able to define differences within the microhabitat of each species or to identify differences between individuals during development. Although studies of intraspecific ontogenetic variation have not been performed, variation in leg membrane relative size does not appear to be related to body size, with larger animals having larger variances, as one might expect if there

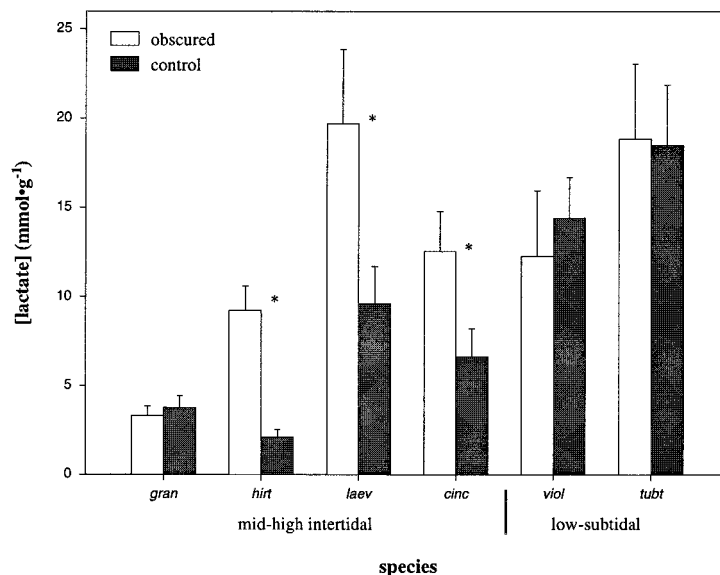


Figure 4. Analysis of leg membrane functional diversity in species from different vertical zones and with different metabolic demands. Whole-animal lactate accumulation following 5-h emersion periods at moderately high temperatures in six species of *Petrolisthes*. Species are coded as follows: *gran* = *Petrolisthes granulosus*; *hirt* = *Petrolisthes hirtipes*; *laev* = *Petrolisthes laevigatus*; *cinc* = *Petrolisthes cincipes*; *viol* = *Petrolisthes violaceus*; *tubt* = *Petrolisthes tuberculatus*. Error bars are 1 SEM, and $n = 5$ for all species except *P. laevigatus*, where $n = 4$. Asterisks denote a statistically significant difference between lactate accumulation of specimens with leg membranes obscured and controls (ANOVA, $P < 0.05$). Species are arranged by microhabitat and by metabolic demands, as indexed by size and activity level; high intertidal zone, low metabolic rate is toward the left, and low intertidal zone, high metabolic rate is toward the right. Data for *P. cincipes* are from Stillman and Somero (1996).

were environmentally induced differences in leg membrane relative size (Fig. 2A–2C). Assuming that the variation is genetic and not created by environmentally induced ontogenetic differences, then the potential for natural selection to produce variation in leg membrane relative size exists (Feder 1987).

Maximum body size in species that possess leg membranes ranges from ~5 to ~30-mm carapace length. This size range represents almost the entire size range for all of the species within this genus and for almost all of the species within the entire family Porcellanidae. While this size range is small when compared to classical studies of physiological scaling (e.g., the “shrew to elephant” curve of size-specific oxygen consumption rates [Schmidt-Nielson 1991]), it is sufficient to see a positive relationship between leg membrane relative size and body size (Fig. 3A–3D).

Functional Significance of Leg Membranes

Measurement of whole-organism lactate levels following a 5-h aerial incubation of six species of *Petrolisthes* (Fig. 4) indicated that possessing leg membranes allowed intertidal species (*Petrolisthes hirtipes*, *Petrolisthes cincipes*, and *Petrolisthes laevigatus*) to remain aerobic while emersed, but that low-intertidal and subtidal species (*Petrolisthes violaceus* and *Petrolisthes tuberculatus*) were unable to remain aerobic (Fig. 4). In addition,

in the small-bodied *Petrolisthes granulosus* (Table 1), whose leg membranes are small (Fig. 1B; Table 1), the obstruction of the leg membranes had no effect on lactate accumulation (Fig. 4). Thus, the functional capabilities of the leg membranes of intertidal species appear to be adequate in supporting aerial respiration during low-tide periods. In the low-intertidal and subtidal species, which do not normally experience long periods of emersion, the functional significance of the leg membranes as respiratory structures remains uncertain. More studies of the function of leg membranes of low-intertidal and subtidal species (e.g., *P. violaceus*, *P. tuberculatus*, and *Petrolisthes tuberculatus*; Table 1) as a function of time, temperature, and emersion (as in Stillman and Somero 1996) may provide a greater understanding of the respiratory function of leg membranes in those species. In *P. cincipes*, obstruction of leg membranes did not alter aquatic oxygen consumption rates (Stillman and Somero 1996). However, the role of leg membranes as aquatic respiratory structures may be different in the above mentioned species.

In this article, there is no explicit analysis of the total respiratory surfaces of any of the species or of what proportion of the respiratory demands for any species is contributed by the leg membranes. To make such analyses, precise measurement of the area of all respiratory surfaces in the organism would be required. Measuring the gill surface area in anomuran

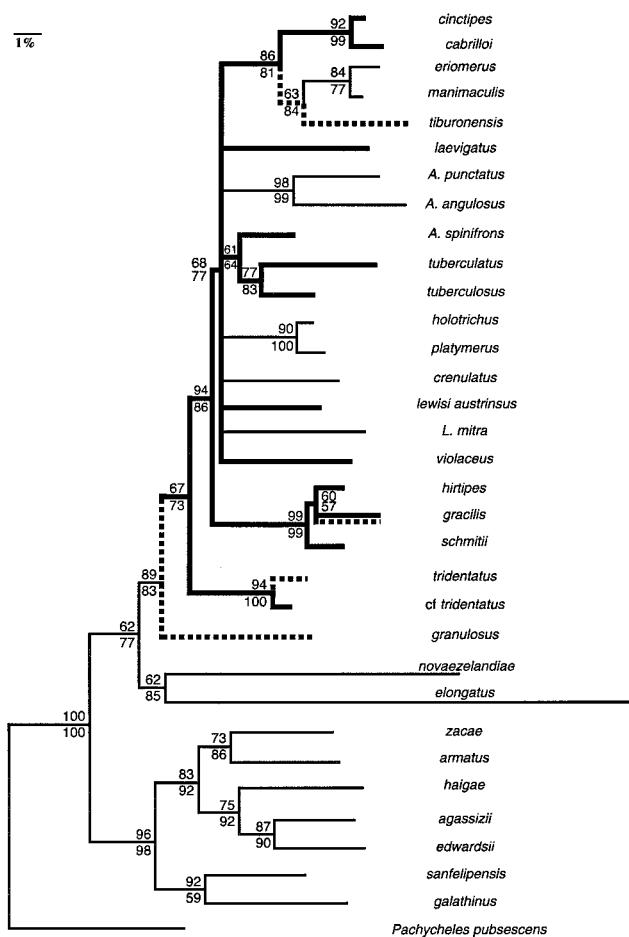


Figure 5. Phylogenetic tree based on the sequence of the 16s rDNA gene showing the evolutionary history of leg membranes. The topology of the phylogenetic tree is based on a distance-neighbor-joining analysis, and bootstrap values from distance (*top*) and parsimony (*bottom*) analyses are shown at each significant node. Species are in the genus *Petrolisthes* except those with generic initial *A* (*Allopetrolisthes*) and *L* (*Liopetrolisthes*) or *Pachycheles*. Thickened lines indicate presence of the leg membrane phenotype; solid lines represent those species that have leg membranes on all three pairs of legs, and dashed lines those species without membranes on leg 1. Phylogenetic analyses were performed using the PHYLIP software package (Felsenstien 1989) as described elsewhere (Stillman 1998).

crabs, such as *Petrolisthes*, is not as straightforward as for brachyuran crabs. This is because, unlike the gills of brachyuran crabs that are arranged into a single compact and geometrically regular set of lamellae per exopodite, the gills of porcelain crabs are arranged into several nonuniform sets of lamellae per exopodite. To make an accurate measurement of the surface area of the gills of a porcelain crab, every lamellar surface would need to be analyzed, while for brachyuran crabs, measuring the area of a single surface from each set of lamellae is sufficient to calculate the total surface area of that set of lamellae. An

ultrastructural analysis of the leg membranes may reveal interspecific differences in the membrane or submembrane vasculature that could affect gas exchange, further altering the functional contribution of the leg membranes.

Evolutionary History of Leg Membranes

The leg membrane phenotype is contained entirely within the clade containing “smooth-bodied” *Petrolisthes*, and it appears to be the ancestral condition for eastern Pacific “smooth” species (Fig. 5, *thick solid* and *dashed* lines). In addition, the leg membrane may have first arisen only on legs 2 and 3 and have been small in size, if the phenotype of *P. granulatus* is similar to the ancestral condition (Fig. 5, *thick dashed* lines; Table 1). The phenotype of absence of leg membranes on leg 1 in other species (*Petrolisthes tiburonensis*, *Petrolisthes gracilis*, and *Petrolisthes tridentatus*) represents a reversion back to ancestral conditions (Fig. 5, *thick dashed* lines).

The relationship between leg membranes and vertical intertidal zone distribution is somewhat clarified by these phylogenetic analyses. *Petrolisthes granulatus* lives high in the intertidal zone (Table 1), suggesting that semiterrestrial microhabitat conditions may be ancestral to the eastern Pacific “smooth” species. Loss of leg membranes has occurred in some species that are primarily subtidal (Haig 1960; Stillman 1998), such as *Petrolisthes eriomerus*, *Petrolisthes manimaculis*, *Petrolisthes crenulatus*, *Allopetrolisthes punctatus*, and *Liopetrolisthes mitra* (Fig. 5). Loss of leg membranes has also occurred in some

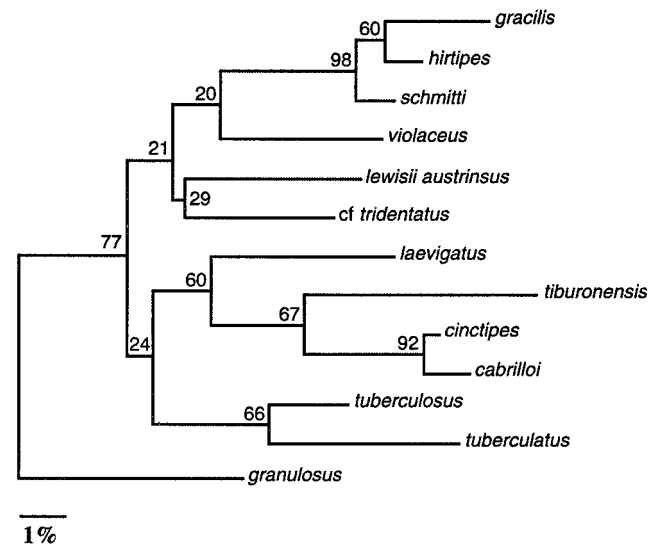


Figure 6. Phylogenetic tree that was used for the generation of independent contrasts. These 13 species of *Petrolisthes* are those that possess leg membranes and for which data were available on leg membrane relative size, intertidal zone distribution, and 16s rDNA sequence. Phylogenetic analyses were performed as in Figure 5.

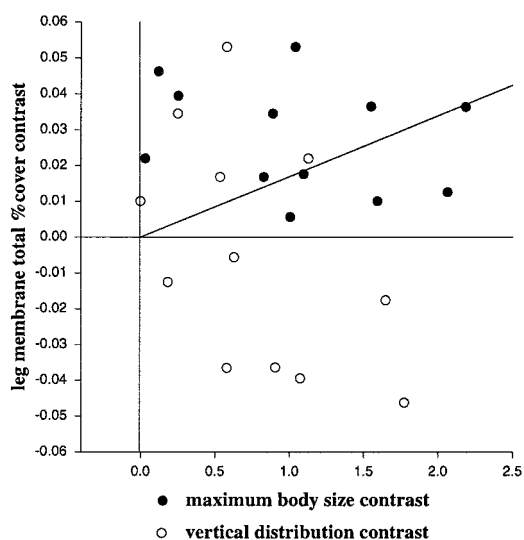


Figure 7. Adaptive significance of leg membranes. Independent contrasts of leg membrane relative size plotted against independent contrasts of maximum body size and vertical distribution. Regression analysis of leg membrane coverage contrast versus maximum body size contrast indicated a positive correlation between these variables (solid line, $Y=0.017X$). Regression analyses of leg membrane relative size contrast versus vertical position contrast did not indicate a significant relationship between the two variables, and thus no line is shown.

middle intertidal species, such as *Allopetrolisthes angulosus*, *Petrolisthes holotrichus*, and *Petrolisthes platymerus*, the latter two of which are small in size (Table 1). Leg membranes have been retained in some species that live in the low intertidal or subtidal zones, such as *P. violaceus*, *Allopetrolisthes spinifrons*, *P. tuberculatus*, and *P. tuberculosus* (Table 1).

Adaptive Significance of Leg Membranes

The statements in Stillman and Somero (1996) were conservative regarding the adaptive significance as an aerial respiratory structure of the leg membranes in *P. cinctipes*. Assuming that *P. granulatus* is similar in form and physiology to the ancestral species in which leg membranes first arose, then it is difficult to ascribe the term “adaptation” for aerial respiration to the leg membrane phenotype. *Petrolisthes granulatus* lives high in the intertidal zone but has small leg membranes that do not play a large functional role during aerial respiration (Figs. 1, 4; Table 1) and may not offer any fitness advantage. In the larger-bodied intertidal species (e.g., *P. cinctipes*, *P. laevigatus*) leg membranes might most accurately be labeled “exaptations” for aerial respiration, as the leg membranes are functionally significant for aerial respiration in these species but did not evolve specifically as aerial respiratory structures. Independent contrasts analysis does not indicate any evolutionary relationship between leg membrane relative size and vertical position

in the intertidal (Fig. 7, open symbols), further reducing the argument that leg membranes are “adaptations” for aerial respiration.

Independent contrasts analysis indicates that there is an evolutionary relationship between leg membrane relative size and body size (Fig. 7, filled symbols). Assuming that the ancestral condition of “smooth” *Petrolisthes* includes small body sizes and small leg membrane relative sizes (i.e., similar to *P. granulatus*; Figs. 5, 6; Table 1), the presence of leg membranes may have facilitated the evolution of increased body size in intertidally living species. In this context, leg membranes may provide the supplemental aerial respiratory surface needed for the increasing metabolic demands associated with larger body sizes of intertidally living species.

Some of the largest (in terms of carapace dimensions) *Petrolisthes*, such as *P. violaceus* (Table 1), are dorso-ventrally expanded, thereby increasing their body volume by a disproportionate amount to the increase in body surface area. The increased whole-organism oxygen demands that results from possessing additional body tissue requires a compensatory change in the ability of respiratory structures to acquire sufficient oxygen to prevent a state of metabolic hypoxia. The presence of leg membranes may provide sufficient additional respiratory surface area to allow these species to remain aerobic. In *P. violaceus*, the leg membranes in this species may provide enough of an accessory respiratory surface to offset the increases in volume, allowing larger body volumes to evolve.

Two species of *Petrolisthes*, *P. tuberculatus* and *P. tuberculosus*, are extremely active, with locomotor activities that are more similar to grapsid crabs such as *Grapsus grapsus* or *Pachygrapsus crassipes* than to other porcelain crabs (J. H. Stillman, personal observation). These species are both large (in terms of carapace dimensions; Table 1), but unlike *P. violaceus*, are more dorso-ventrally flattened than most other species of *Petrolisthes* (J. H. Stillman, personal observation). The large leg membranes of these species may provide the necessary accessory respiratory surface to support the higher metabolic demands of increased locomotor activity, allowing these crabs to evolve more rapid movement. Further experimentation on the role of the leg membranes in supporting exercise-based metabolic demands is clearly required before any firm conclusions can be made.

One caveat to these arguments of the adaptive significance of leg membranes stems from the assumption that ancestral character states are similar to those observed in extant species. *Petrolisthes granulatus*, although being most similar to the ancestral species in genotype, may not be similar in phenotype. The leg membrane phenotype has been lost several times within the genus, where the phenotype of species having large leg membranes on all legs changes to species with no leg membranes (Fig. 5). The amount of genetic divergence from *P. granulatus* to its closest ancestor is ~7% (Figs. 5, 6). This is a much larger value than for the amount of genetic divergence (~1.5%) from the common ancestor of *P. eriomerus* and *P.*

manimaculis (two species that lack leg membranes) to its nearest ancestor that possesses membranes on all legs (Fig. 5). Assuming that genetic mutations accumulate at an equal rate among taxa, *P. granulatus* would have had three to four times as long to reduce their leg membranes than it took for the common ancestor of *P. eriomerus* and *P. manimaculis* to completely lose their leg membranes. Thus, the ancestral condition might have been large leg membranes. Only fossil evidence, which has not been reported for *Petrolisthes*, can resolve this issue.

Leg Membranes—Convergent Evolution

Leg membranes have been described from two distantly related groups of crabs: the brachyuran Ocypodid ghost crabs, in the genera *Scopimera* and *Dotilla* from Australia (Maitland 1986), and the anomuran Porcellanid porcelain crabs of the eastern Pacific (Jensen and Armstrong 1991; Stillman and Somero 1996). In addition to the previously reported cases, membranous regions can be found on the limbs of additional brachyuran crabs, including other Ocypodid crabs, such as *Scopimera bitympana* (photograph in Wang and Liu 1996), where the membranes are located on both sides of the meral segment of walking legs. The Portunid crab *Callinectes sapidus* may have leg membranes on every segment of their legs, although comparison with other Portunid crabs is necessary to confirm the thin exoskeleton as a leg membrane. Leg membranes also have been observed in other anomuran crabs, such as the Hippid mole crab, *Emerita analoga*. In addition, membranous regions have been described on other regions of anomuran crabs, such as the hydrothermal vent Galatheid crab, *Munidopsis lentigo*, where on the dorsal surface of the manus of each cheliped there is a small decalcified spot (Williams and Van Dover 1983). Further studies to examine the functional significance of the membranous regions of these crabs, as well as to determine their evolutionary histories may advance our understanding of the adaptive significance of membranous decalcified areas in decapod crustaceans.

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