AN ABSTRACT OF THE THESIS OF

Joshua J. Borland for the degree of Honors Baccalaureate of Science in Biology (Honors Scholar) presented on May 20th 2014. Title: Evidence for the Potential Hybridization of *Ammophila breviligulata* and *Ammophila arenaria* in the Pacific Northwest.

Abstract approved: ______________________________

Sally D. Hacker

Coastal dunes are dynamic and unique ecosystems. On the Pacific coast, dunes are generally dominated by the beach grasses, *Ammophila breviligulata* and *Ammophila arenaria*, two invasive species introduced in the early 1900's to stabilize sand and create foredunes that serve to protect people who live behind them. Recently, a novel variant of *Ammophila*, a potential hybrid of the two congeners, has been observed on the coast and this project was aimed at investigating the possible source of this unique grass. To investigate, I measured morphological traits among four different grasses, *A. arenaria*, *A. breviligulata*, *Elymus mollis* and the potential hybrid. The potential hybrid was shown to be statistically different in morphology when compared to the other *Ammophila* species, and the native out-group *E. mollis*, including; intermediate ligule length, number of tillers per plant, and above ground biomass but greater tillers per m^2, tiller length, and below ground biomass. Further studies are needed on the presumed hybrid to confirm its genetic background, as well as its potential effects on the dune ecosystem. In particular, can it compete with its parent or native species and will it change the shape of the dune through biophysical feedbacks, thus altering coastal protection.

Key Words: Hybridization, dune grass, morphology, *Ammophila*

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Evidence for the Potential Hybridization of *Ammophila breviligulata* and *Ammophila arenaria* in the Pacific Northwest

by

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I understand that my project will become part of the permanent collection of Oregon State University, University Honors College. My signature below authorizes release of my project to any reader upon request.

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Joshua J. Borland, Author

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First, I would like to thank Sally Hacker, my Mentor, for agreeing to take me on and helping to direct me towards a worthy and interesting research project. Furthermore, I would like to thank Peter Ruggiero for being on my committee and helping to bring my thesis to fruition. I would also like to thank Reuben Biel for the hours he spent helping me with collection and statistical analysis. I feel he went above and beyond and I hope this thesis is a small reward. Last I would like to thank my family and fiancé for supporting me on my journey through the education process. They have been a powerful force pushing me towards success and happiness.

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Introduction:

Arguably one of the largest ecosystems along the Oregon and Washington coasts is sandy beaches and dunes. Pacific Northwest dunes are widespread and cover variable extents, from a few kilometers to stretches as long as 60 km (Wiedemann and Pickart 1996). Historically, the native *Elymus mollis* was the dominant beach grass and it created small hummocks with loose sand (Cooper 1958). The limited stabilization provided by *E. mollis* allowed for sand movement, which was a concern for coastal communities because it encroached on and covered towns and roads, and created the potential for dune blowouts. Aside from limiting sand movement, stabilized beaches and dunes provide protection against wave damage and are valuable tourist destinations (Whitfield and Brown 1948, Barbier et al. 2011).

To reduce sand movement, *Ammophila arenaria* was introduced as early as 1896 as part of an effort to stabilize the dunes (Weintraub 1953). This introduction was part of an experimental study of 75 species for survivability on the Oregon coast (Green 1965). In 1935, *Ammophila arenaria* and *Ammophila breviligulata* were used in the first large scale project of intentional planting to stabilize the dunes as part of a two-step program led by the Soil Conservation Service to eventually create the dunes (Whitfield and Brown 1948). Consequently, *E. mollis* has been replaced by the co-dominants *A. arenaria* and *A. breviligulata*, and these are generally the grasses we see today. More importantly, we have seen a shift in dune structure away from small hummocks to large stable foredunes (hills of sand parallel to the shoreline) that vary in shape as a result of the dominance transition (Hacker et al. 2012).

These introduced grasses highly modify their habitat by capturing sand and creating foredunes, which further interrupt sand delivery to the backdune (Hacker et al. 2012). *A. breviligulata* originates on the eastern coast of the United States where it plays an important role in coastal protection via dune building. *A. arenaria* plays a similar role but originates from Europe. Both are known to spread via rhizome extension as well as sexually via seed production, though seeds rarely establish successfully (Wiedemann 1987). Where they have been introduced and subsequently established on the Pacific coast, foredune height varies from 1.4 to 13.7 m above the foredune toe (Seabloom et al. 2013). Those dominated by *A. breviligulata* are shorter and wider than those dominated by *A. arenaria* (Hacker et al. 2012, Zarnetske et al. 2012). This is due, in part, to the way in which the different grass species grow, with *A. arenaria* growing vertically and densely versus *A. breviligulata* growing more laterally and less densely. While dune shape differs depending on which grass dominates the dune, a large part of the dune structure is determined by sand supply, with high rates of sand supply resulting in wider dunes and low rates of sand supply resulting in taller dunes (Hacker et al. 2012).

Over the last few years, R. Biel and S. Hacker in the Department of Integrative Biology at Oregon State University, and A. David in the Department of Ecology, Evolution, and Behavior at the University of Minnesota, observed grasses in several Oregon and Washington locations that had intermediate morphological characteristics between *A. arenaria* and *A. breviligulata*. In particular, they notice that the ligule (a thin outgrowth of tissue between the leaf and leafstalk often used to identify species) of the unusual grass was intermediate in length between the two *Ammophila* congeners. This “blending” of traits led to the hypothesis that this grass might be a hybrid between the two *Ammophila* species. Because grass morphology has a large effect on dune shape (Hacker et al. 2012, Zarnetske et al. 2012), this hybridization event could have a large impact on the functions and services provided by this ecosystem (Ayres et al. 2008). For example, depending on the sand capturing ability of the hybrid, we might expect the dune structure to change. This could negate efforts to stabilize the dune or result in more extreme dune shapes. Furthermore, this hybrid could out-compete its parent plants or native plants. However, before studies of ecosystem effects can be conducted, it needs to be determined if the observations of different morphology for the presumed hybrid are in fact different than the parent plants. To do so, an analysis of the intermediate ligule morphotype was conducted to see if the morphological inconsistency is within natural variation or is distinct. To do so, I compared the morphological traits between the hypothesized non-native parents (*A. breviligulata*, and *A. arenaria*), a third native dune grass, *Elymus mollis*, and the potential *Ammophila* hybrid.

Material and Methods:

Surveys:

A survey of *A. arenaria*, *A. breviligulata*, *E. mollis*, and the potential hybrid was conducted along the northeast Pacific coast in Grays Harbor, WA (-124.114, 46.85235), Long Beach, WA (-124.059, 46.51255 and -124.059, 46.43369), and Pacific City, OR (-123.968, 45.20274) (Table 1, Figure 1). We conducted the surveys in areas along the beach that were observed to contain all the species of interest and each location was identified and recorded using GPS. We also measured tiller density of each species using a standard 0.5 m x 0.5 m quadrat and collected a minimum of 20 plants from each species and site. In total, we collected 88 plants of *A. arenaria*, 101 plants of *A. breviligulata*, 105 plants of the hybrid, and 49 plants of *E. mollis* (fewer plants were collected because of the low abundance of this species on the beach).

Plant measurements:

After collections were completed, I measured the samples' morphology to investigate morphological differences between each species and site-to-site variation. These measurements included: the ligule length, number of vertical tillers, number of horizontal tillers, presence or absence of inflorescences, blade length, blade width, tiller length, above ground biomass, below ground biomass, number of leaves, number of rhizome nodes, length of rhizome internodes, and total rhizome length. We normalized aboveground and belowground biomass to determine mean aboveground biomass per tiller and mean belowground biomass per cm of rhizome length. Vertical tillers are the shoots visible from the surface of the ground. The horizontal tillers are branches of the root system that lead to additional clusters of vertical tillers and these occur at right angles from the rhizome. Tiller length was measured from the primary node to the tip of most central blade, blade length was measured from the primary node to the tip of the longest blade that was not the most central, and ligule length was measured from the base of the blade, most easily seen from the side or internal face of the blade, to the tip of the ligule (Figures 2, 3). We avoided measuring the same blade for blade length and tiller length.

The tiller length, blade length, internode length, and rhizome length were measured to the nearest 0.5 centimeter, and the blade width and ligule length were measured to the nearest millimeter. Biomass was measured to the nearest tenth of a gram.

Statistics:

I performed all statistical analyses using R 3.1.0 (R Development Core Team, 2013). To evaluate morphological differences between the species, I performed multiple linear regressions to compare tiller length, blade width, leaf length, ligule length, below ground, and above ground biomass to species identity and site [example R code: lm(ligule_length ~ Species + Site)]. Furthermore, I performed a Poisson regression to compare tiller number and leaf number between species and site. Finally, I used Akaike Information Criteria (AIC) to identify the optimal model.

Results:

Density

Some of the tiller density survey data for the four grasses was misplaced. Of the data that was not lost, the potential hybrid tiller density was determined to be 452.0 ± 23.6 m^2 (n = 3). The data for the potential hybrid, combined with tiller density data from Zarnetske et al. (2012), suggests that the hybrid had higher tiller densities than the presumed parents or the native, *Elymus mollis* (Table 2).

Morphology

The hypothesized *Ammophila* hybrid exhibited multiple distinct morphological traits that were significantly different from both parent *Ammophila* species and the native grass, *E. mollis* (Table 3). The potential *Ammophila* hybrid exhibited a mean ligule length of 7.2 ± 0.01 mm (mean ± SE) (Figure 4; Table 3). We found that ligule length significantly differed between species, with the potential hybrid being on average 21 mm shorter than *A. arenaria* and 6 mm longer than *A. breviligulata* (p-value < 0.0001) (Figure 4; Table 3). Furthermore, the hybrid had a mean blade width of 6.9 ± 0.02 mm, which was larger on average (1.8 mm) wider than *A. arenaria* and *A. breviligulata* (0.6 mm) (p-value < 0.0001) (Figure 5; Table 3). Blade width was also significantly different across sample site with Long Beach being on average 0.85 mm wider (p-value < 0.0001) and Pacific City 0.67 mm narrower (p-value = 0.003) than at Grays Harbor. The hybrid tiller length had a mean of 84.3 ± 0.26 cm and was different across species with the potential hybrid being on average 12.6 cm longer than *A. arenaria* and 13 cm longer than *A. breviligulata* (p-value < 0.0001) (Figure 6; Table 3). Similarly, the hybrid leaf length had a mean of 103.1 ± 0.23 cm and was significantly different across species with the potential hybrid being on average 15.8 cm longer than *A. arenaria* and 15.7 cm longer than *A. breviligulata* (p-value = 0.007) (Figure 6; Table 3).

The hybrid had a mean of 3.4 ± 0.03 vertical tillers per plant and was significantly different across species with the potential hybrid having on average 1.7 less tillers per plant than *A. arenaria* and 0.9 more than *A. breviligulata* (p-value < 0.0001) (Table 3). The number of leaves is also significantly different across species with the potential hybrid having a mean of 4.8 ± 0.02 leaves with on average 1.5 more leaves than *A. arenaria* and 1.2 less than *A. breviligulata* (Table 3).

Biomass

The above ground biomass was normalized by tiller count to produce mass per tiller, and below ground biomass was normalized by rhizome length to produce mass per unit length. The above ground biomass differences were significant across species with the potential hybrid having a mean of 3.14 ± 0.02 g per tiller and was an average 1.7 g heavier than *A. arenaria* and 0.7 g lighter than *A. breviligulata* (p-value < 0.0001) (Figure 7; Table 3). Below ground biomass was also significantly different across species with the potential hybrid having a mean of 0.26 ± 0.01g per unit rhizome length and was on average 0.17g heavier that *A. arenaria* and 0.2 g heavier than *A. breviligulata* (p-value < 0.0001) (Table 3). Total biomass was also significantly different across species with the potential hybrid having a mean of 9.3 ± 0.09 g and was on average 2.6 g heavier than *A. arenaria* and 0.5 g heavier than *A. breviligulata* (Table 3).

Discussion:

The potential hybrid is distinct from the other two non-native beach grasses suggesting that this grass may be a hybrid of the two species. Multiple traits, including ligule length, blade width and others, help to confirm that the trait variability is outside of what we would expect in natural variability of the two *Ammophila* congeners. One or two traits would suggest a new morphological population of either *A. arenaria* or *A. breviligulata*, but the combined total of varied traits is more indicative of hybridization. It is important to note, while some of the traits we measured showed intermediate form, many were more extreme than both parents, including tillers per m^2. This could be indicative of hybrid vigor (Baranwal et al. 2012). To further support this conclusion genetic comparison of the hybrid and parental species should be conducted. It was outside the scope of this project but previous studies have shown the *A. breviligulata* is genetically diverse, which could lead to problems of identifying possible genetic markers (Slaymaker et al. 2013). However, *A. arenaria* has hybridized in the past supporting the possibility that it has hybridized again (Rihan and Gray 1985).

Exploring the significance of the hybridization event will be an important topic of future studies. Previous studies have shown that hybrids can outperform either of their parents. This potentially higher fitness could be the result of ecological factors including predatory resistance or predator avoidance, due to less palatable hybrids (Grosholz 2010, Krebs et al. 2011), or indirect competition (Larkin et al. 2012). When *A. arenaria* was introduced into the Florence, OR, region it was a genotype from Coos Bay, OR, which was, in turn, transplanted from San Francisco, CA. The San Franscisco plants were introduced from Australia, where they had been originally transported from Europe. This multiple population reduction via sample introduction likely has limited the genetic diversity within each resulting location (Figure 8). This reduced genetic diversity could result in inbreeding depression which has been shown to reduce total fitness. The effect of the inbreeding depression can be reduced by introduction of new genetic material, for example, as in a hybridization event (Hedrick and Kalinowski 2000). The reduced fitness of *A. arenaria* and/or *A. breviligulata* combined with increased fitness of the hybrid and natural selection can lead to a positive feedback loop of invasion potential, with the hybrid continuing to outcompete native plants as well as its introduced parental species (Ayres et al. 2008).

It has also been documented that once an invasive hybrid population is established it can be an uphill battle to prevent further spread or to restore original native ecosystems. One removal effort in 1994 specifically involving the *Ammophila* genus cost $20,000 per ha, which highlights the potential financial burden faced due to inaction (Wiedemann and Pickart 1996). However, with all of this in mind there is hope. There have been successful programs focused on illuminating potentially hazardous or costly hybrids with aggressive eradication (Ayres et al. 2008).

Aside from invasion risk, there is a potential for the loss of ecosystem services as a result of changing dune structure. The potential hybrid of *Ammophila* might be expected to change foredune shape quite dramatically. Further studies would need to be conducted to see how this hybrid captures sand, responds to sand deposition, and in general how it grows in comparison to *A. arenaria* and *A. breviligulata*. However, from previous studies it might be expected that the longer wider tillers and higher densities will result in taller dunes than either *A. arenaria* or *A. breviligulata* (Zarnetske et al. 2012, Seabloom et al. 2013) (Figure 9). These taller dunes can help to reduce wave-overtopping and further protect the coast, however, houses directly behind these dunes may be displeased as the view of the ocean is diminished behind a wall of sand (Seabloom and Wiedemann 1994). The difference in dune height may also affect native plants and animals, with unforeseen consequences to ecosystem functioning.

Conclusion:

Ammophila breviligulata and *A. arenaria* are cogeneric dune grasses that populate the Pacific coastline since their introduction in the early 20th century. Since then, they have had a long time to co-occur and it appears recently this has resulted in a hybrid plant. The hybrid has been shown to have a blending of a few morphological features in addition to many traits in which the hybrid is larger than either parent. Further studies need to be conducted on the hybrid to confirm its genetic background, as well as to determine its potential effects on the dune ecosystem, whether through competition with its non-native parent species, displacement of the native plant and animal community, and/or the dune building process. Finally, an analysis of its invasion potential should be done to find out if there is concern of widespread effects or if the hybrid will likely be limited to the areas in which it developed.

Bibliography

Ayres, D. R., K. Zaremba, and D. R. Strong. 2004. Extinction of a common native species by hybridization with an invasive congener. Weed Technology **18:**1288-1291.

Ayres, D. R., E. Grotkopp, K. Zaremba, C. M. Sloop, M. J. Blum, J. P. Bailey, C. K. Anttila, and D. R. Strong. 2008. Hybridization between invasive *Spartina densiflora* (Poaceae) and native *S. foliosa* in San Francisco Bay, California, USA. American Journal of Botany **95**:713-719.

Baranwal, V. K., V. Mikkilineni, U. B. Zehr, A. K. Tyagi, and S. Kapoor. 2012. Heterosis: emerging ideas about hybrid vigour. Journal of Experimental Botany **63**: 6309-6314.

Cooper, W.S., 1958: Coastal Sand Dunes of Oregon and Washington, Memoirs of the Geological Society of America, **72:**1-169

Green, D. L. 1965. Deveolopmental history of European beachgrass (*Ammophila arenaria*) planting on the Oregon coastal sand dunes. Oregon State Library **1**:1-64.

Grosholz, E. 2010. Avoidance by grazers facilitates spread of an invasive hybrid plant. Ecology Letters **13**:145-153.

Hacker, S. D., P. Zarnetske, E. Seabloom, P. Ruggiero, J. Mull, S. Gerrity, and C. Jones. 2012. Subtle differences in two non-native congeneric beach grasses significantly affect their colonization, spread, and impact. Oikos **121**:138-148.

Hedrick, P. W., and S. T. Kalinowski. 2000. Inbreeding depression in conservation biology. Annual Review of Ecology and Systematics **31**: 139-162.

Krebs, C., E. Gerber, D. Matthies, and U. Schaffner. 2011. Herbivore resistance of invasive *Fallopia* species and their hybrids. Oecologia **167**:1041-1052.

Larkin, D. J., M. J. Freyman, S. C. Lishawa, P. Geddes, and N. C. Tuchman. 2012. Mechanisms of dominance by the invasive hybrid cattail *Typha* X *glauca*. Biological Invasions **14**:65-77.

R Development Core Team. 2013. R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. www.r-project.org.

Rihan, J. R., and A. J. Gray. 1985. Ecology of the hybrid marram grass x *Calammophila baltica* in Britain. Vegetatio **61**: 203-208.

Seabloom, E. W., and A. M. Wiedemann. 1994 Distribution and effects of *Ammophila breviligulata* Fern. (American beachgrass) on the foredunes of the Washington coast. Journal of Coastal Research **10**: 178-188.

Seabloom, E. W., Peter Ruggiero, S. D. Hacker, J. Mull, and P. Zarnetske. 2013. Invasive grasses, climate change, and exposure to storm-wave overtopping in coastal dune ecosystems. Global Change Biology **19.**:824-832.

Slaymaker, D. H., M. S. Peek, J.Wresilo, D. C. Zeltner, and Y. F. Saleh. 2013. Genetic structure of native and restored populations of American beachgrass (*Ammophila breviligulata* Fern.) along the New Jersey coast. Journal of Coastal Research **00**:1-10.

Weintraub, F. C. 1953. Grasses introduced into the United States. Washington, D.C.: U.S. Dept. of Agriculture, Forest Service

Whitfield, C. J., and R. L. Brown. 1948. Grasses that fix sand dunes. Yearbook of Agriculture **1**:70-74.

Wiedemann, A. M. 1987. The ecology of European beachgrass. Oregon Department of Fish and Wildlife **87**: 1-15.

Wiedemann, A. M., and A. Pickart. 1996. The *Ammophila* problem on the Northwest coast of North America. Landscape and Urban Planning **34**:287-299.

Zarnetske, P. L., S. D. Hacker, E. W. Seabloom, P. Ruggiero, J. R. Killian, T. B. Maddux, and D. Cox. 2012. Biophysical feedback mediates effects of invasive grasses on coastal dune shape. Ecology **93**:1439-1450

Tables:

Table 1. The location and date where samples were collected.

Point Name	Collection Date	Longitude	Latitude
GH18	6/24/2013	-124.114	46.85235
LB14	6/24/2013	-124.059	46.51255
LB09	6/24/2013	-124.059	46.43369
PC04	7/1/2013	-123.968	45.20274

Table 2. Average tiller densities ± SE of *A. arenaria* (AMAR), *A. breviligulata* (AMBR), *E. mollis* (ELMO), and the potential hybrid (HYBR). Densities of AMAR, AMBR, and ELMO taken from Zarnetske et al. 2012.

Species	Tiller count per m^2
AMAR	222.3 ± 15.2
AMBR	123.8 ± 13.3
ELMO	40.9 ± 4.2
HYBR	452.0 ± 23.6

Table 3: Mean ± standard error of the different morphological traits measured across the species of grasses. See Table 2 for species abbreviations.

	HYBR	AMAR	AMBR	ELMO
Ligule length (mm)	7.2 ± 0.012	28.1±0.073	1.06±0.002	1.02±0.002
Blade width (mm)	6.9±0.015	5.1±0.010	7.5±0.017	9.3±0.040
Tiller count	3.4±0.029	5.0±0.032	2.4±0.019	2.6±0.038
Leaf count	4.8±0.022	3.3±0.011	6.0±0.025	4.2±0.024
Tiller length (cm)	84.3±0.262	71.7±0.204	71.3±0.215	73.7±0.389
Leaf length (cm)	103.1±0.226	87.3±0.105	87.4±0.171	96.1±0.460
Above ground biomass (g) per tiller	3.1±0.021	1.4±0.005	3.8±0.023	4.3±0.046
Below ground biomass (g) per rhizome length (cm)	0.26±0.008	0.10±0.001	0.24±0.003	0.27±0.004
Total above ground biomass (g)	9.3±0.086	6.7±0.041	8.7±0.100	9.6±0.114

Figures:

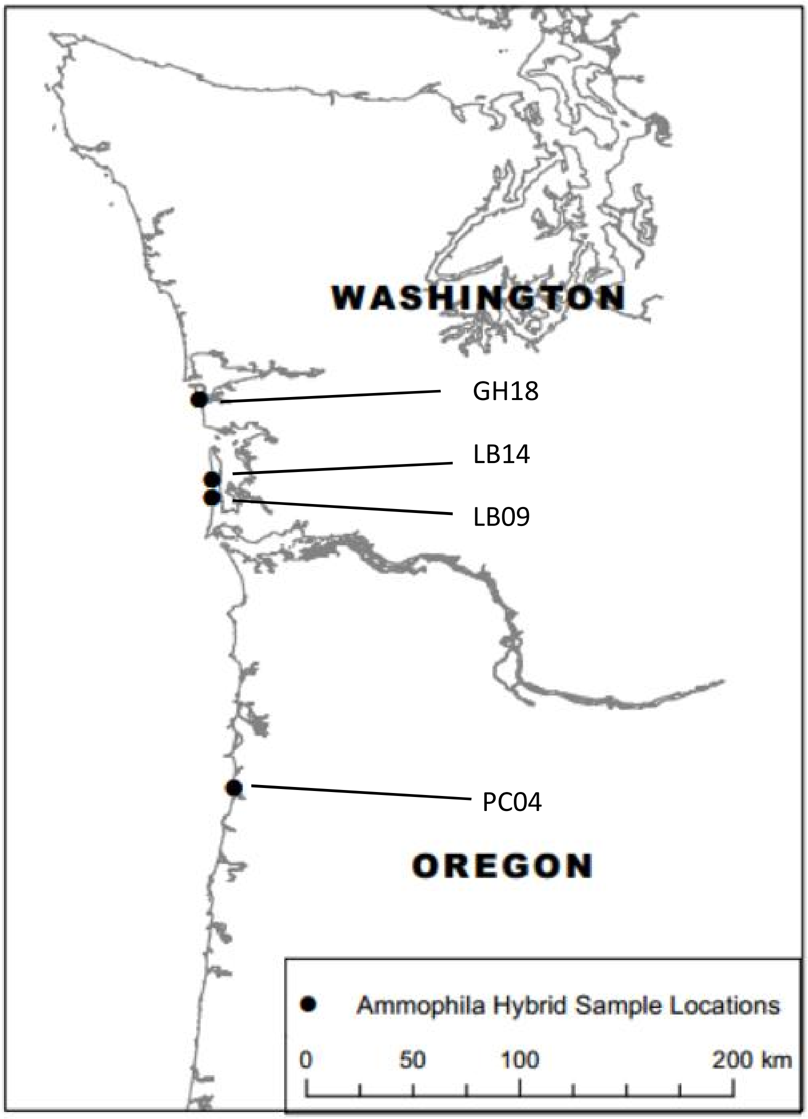

Figure 1: The locations from which the samples were collected: one collection from Grays Harbor WA (GH18), two from Long Beach WA (LB14, LB09), and one from Pacific City OR (PC04).

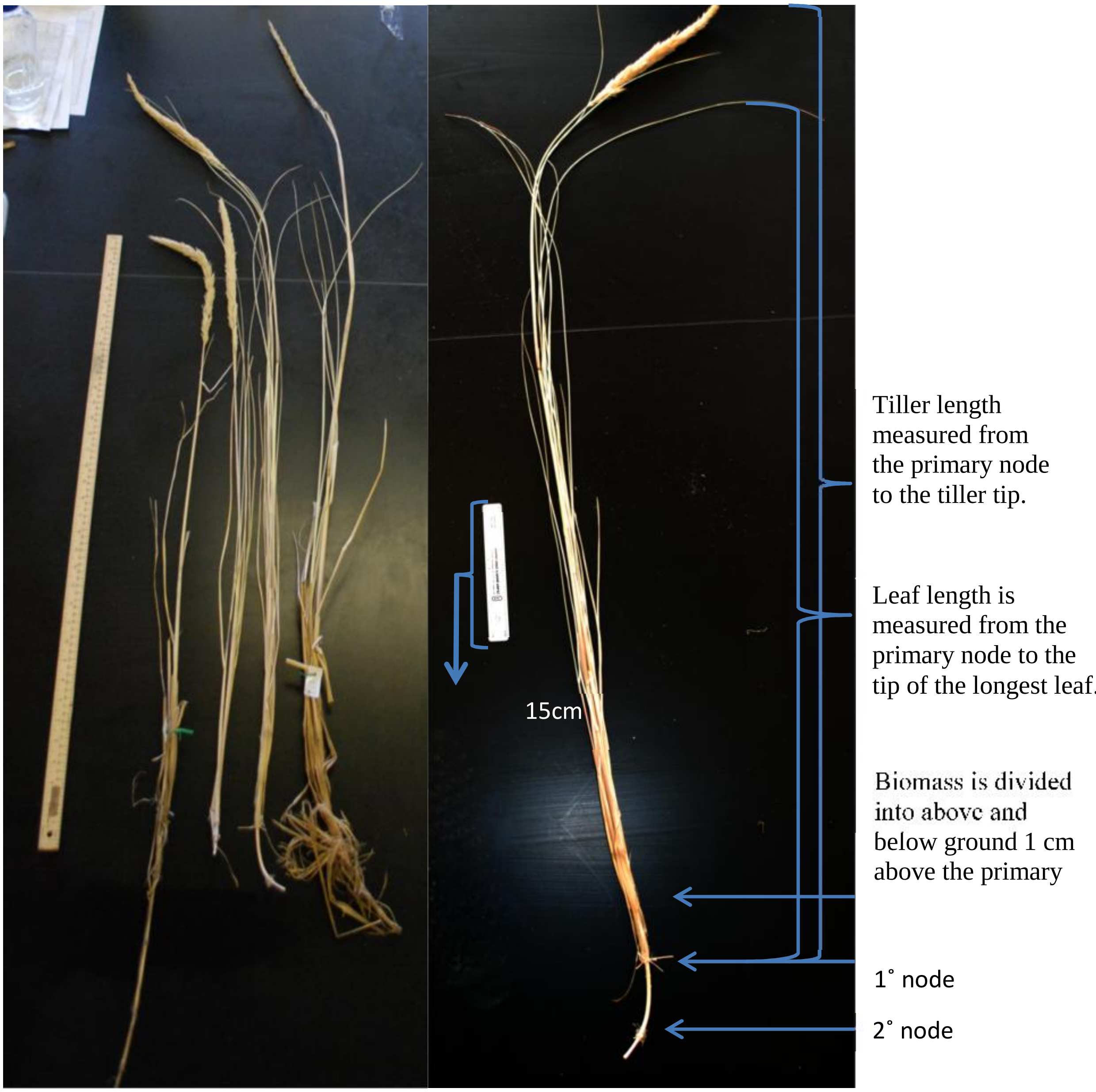

Figure 2. Photo (left) of the beach grass species. They are, from left to right, AMAR, AMBR, HYB, and ELMO. Photo (right) shows the morphological traits measured in the study. See Table 2 for species abbreviations.

Figure 3. The blade width and ligule length of a) *E. mollis*, b) *A. arenaria*, c) *A. breviligulata*, and d) potential *Ammophila* hybrid.

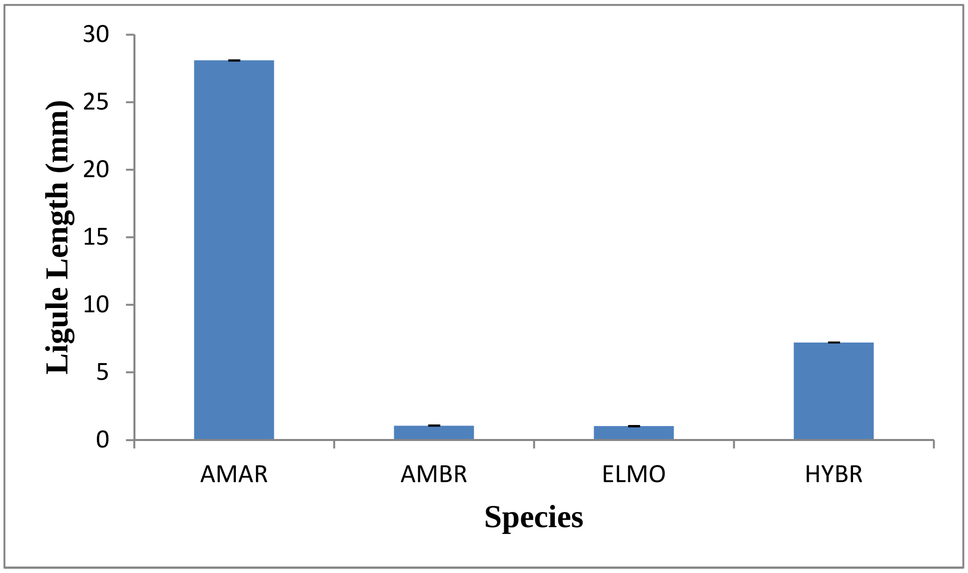

Figure 4. Mean ligule length measured amongst the beach grass species. See Table 2 for species abbreviations.

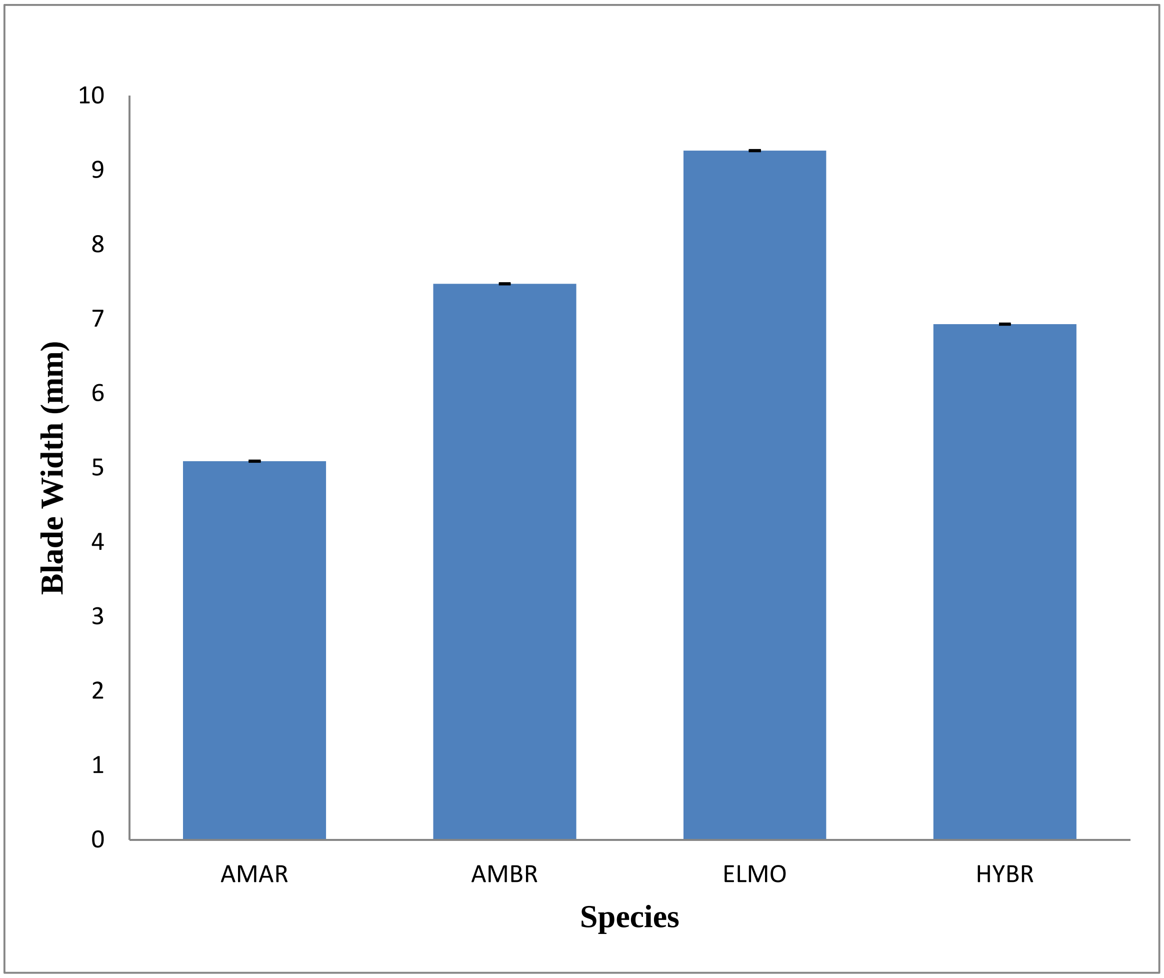

Figure 5. Mean blade width amongst the different beach grass species. See Table 2 for species abbreviations.

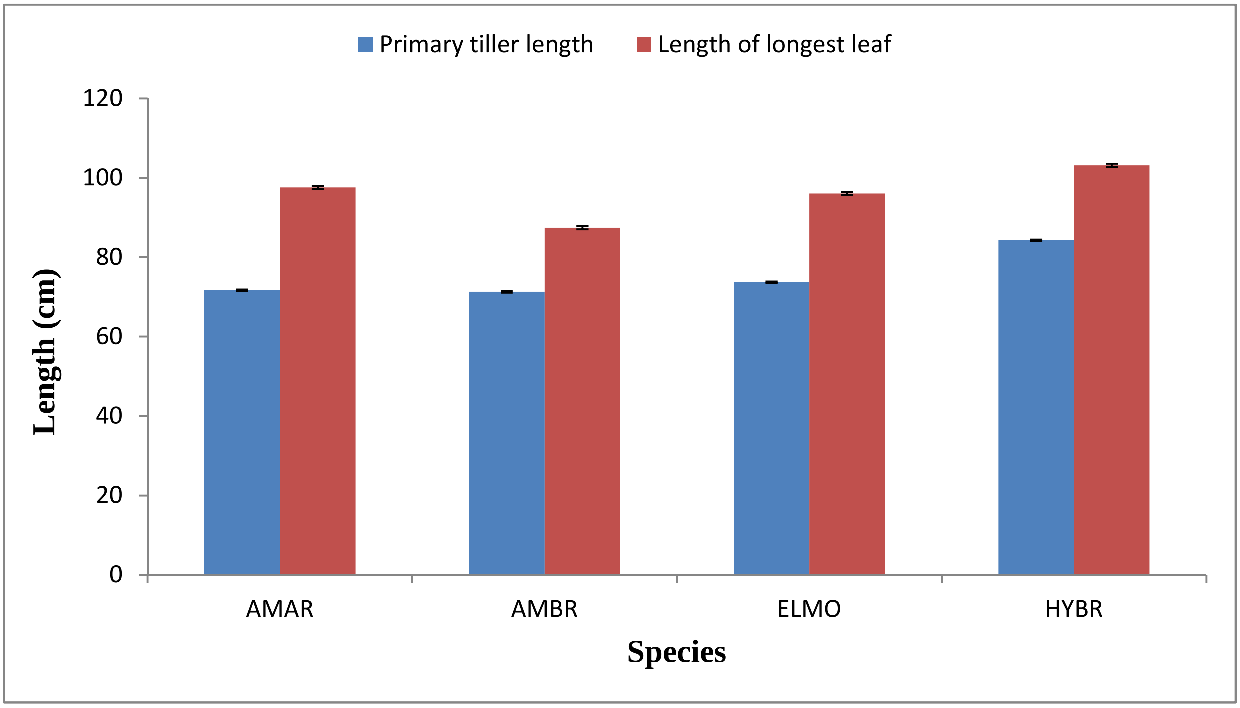

Figure 6. Mean length of the primary tiller and the longest leaf amongst the beach grass species. See Table 2 for species abbreviations.

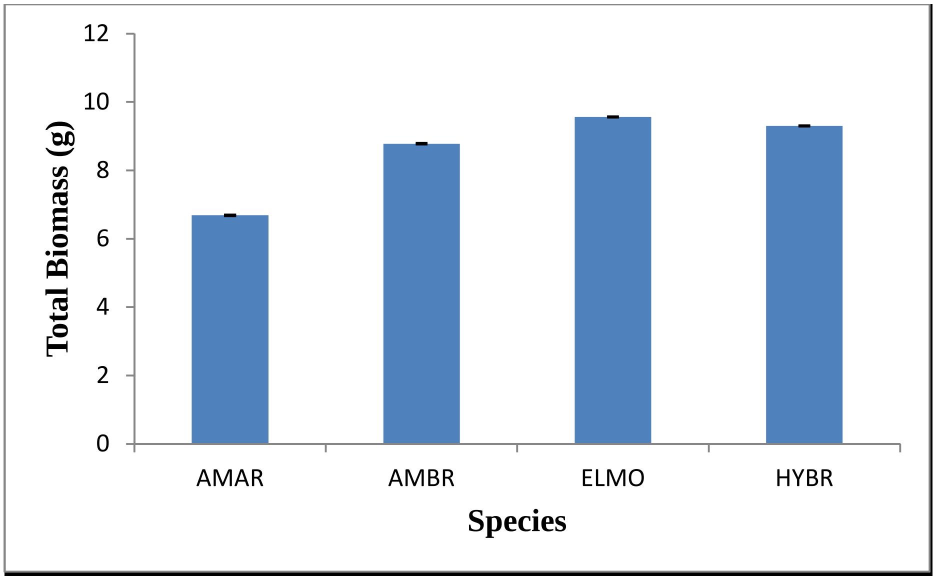

Figure 7. Mean total above ground biomass amongst the beach grasses. See Table 2 for species abbreviations.

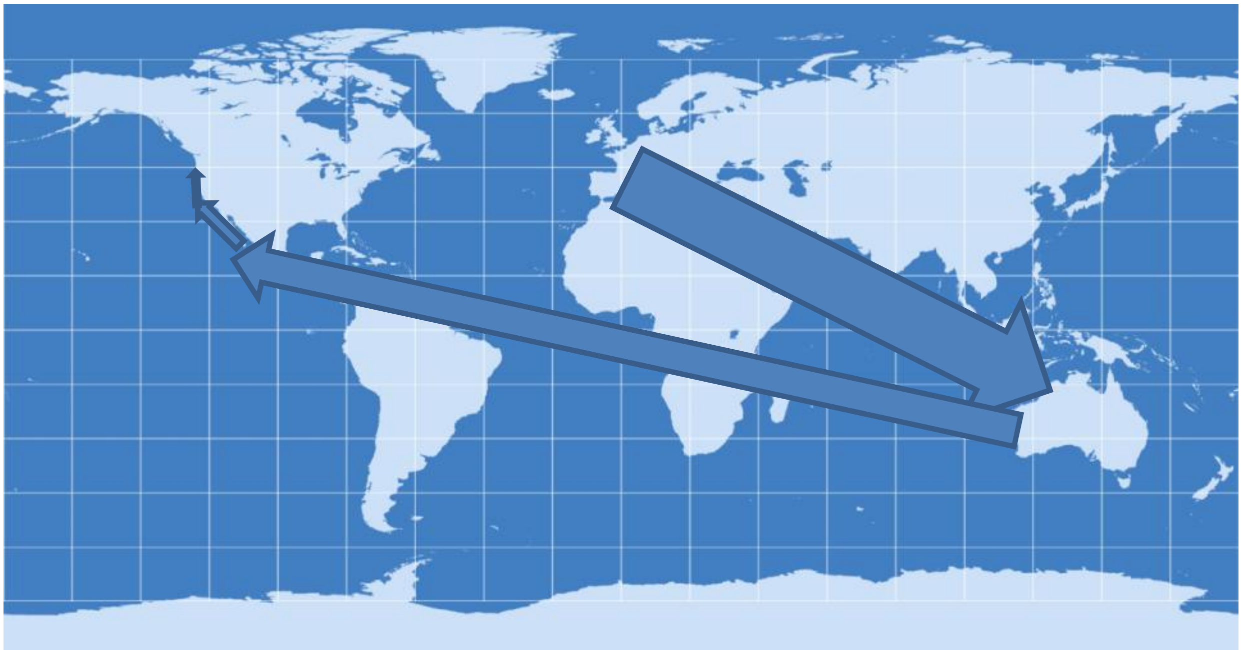

Figure 8. The hypothesized reduction of genetic diversity (represented by arrow width) each time *Ammophila arenaria* was transplanted to areas around the world.

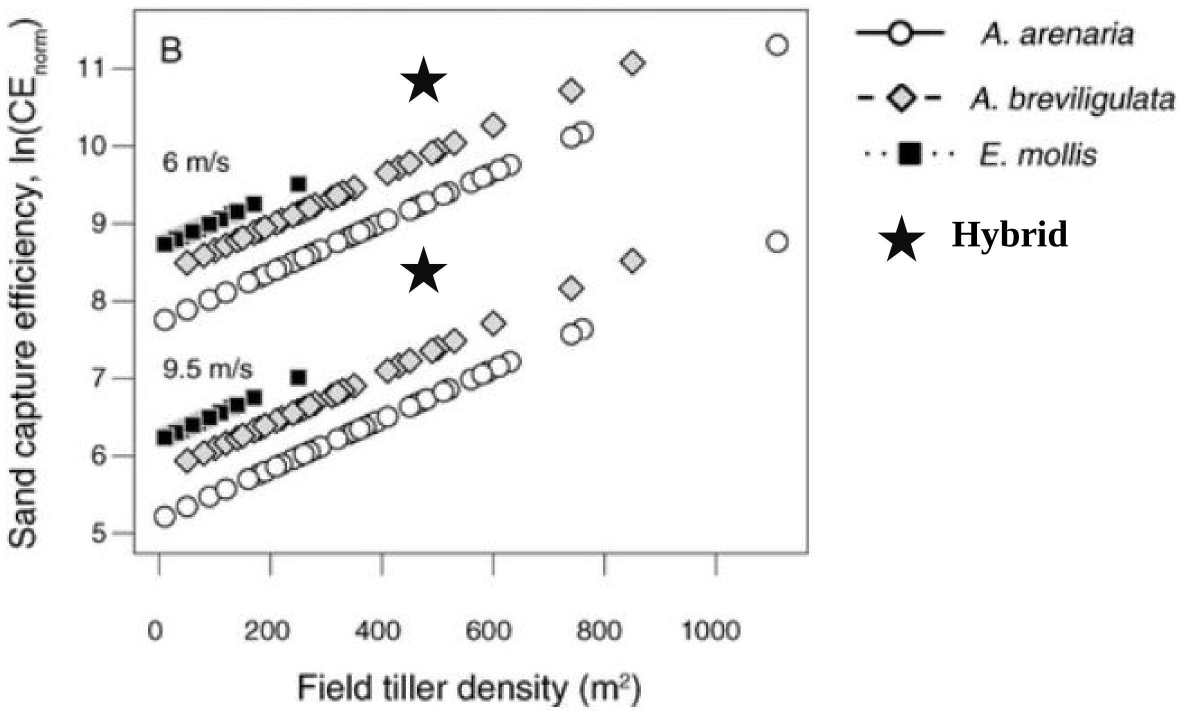

Figure 9. Extrapolation of sand capture efficiency for the potential hybrid based on average tiller density (figure modified from Zarnetske et al. 2012).