DIFFERENTIAL PHYTOSOCIOLOGICAL INTERACTIONS INVOLVING MALE AND FEMALE *ATRIPLEX BONNEVILLENSIS*

Jordan Sinclair^{1,4}, John M. Emlen², Matthew Rinella³, Jessica Snelgrove¹, and D. Carl Freeman¹

ABSTRACT.—Wind-pollinated dioecious plants often exhibit spatial segregation of the sexes. This partial niche separation has most often been explored using abiotic niche axes. However, if the sexes are truly separated in space, then they are apt to encounter different plant species that may heavily affect growth and reproduction. Also, to the extent that their niches differ, the sexes may respond differently to the same co-occurring species. Here we examine interspecific interactions that influence male and female reproductive potential in *Atriplex bonnevillensis*. Using Emlen's interaction assessment, a technique which assesses species interactions based on cover classes, we show that *Salsola* species compete significantly with females but not males, while *Halogeton glomeratus* competes with males but not females. The effect of competition only became apparent when we corrected for site-specific fertility. These results imply that differential competition must be considered when studying dioecious plants that display spatial segregation of the sexes.

Key words: differential competition, spatial segregation of sexes, dioecious.

Many dioecious plant species exhibit spatial segregation of the sexes (Davey and Gibson 1917, Gregg 1975, Freeman et al. 1976, Grant and Mitton 1979, Cox 1981, Fox and Harrison 1981, Wade 1981, Barrett and Thomson 1982, Waser 1984, Vitale and Freeman 1986, Lovett et al. 1987, Dawson and Bliss 1989, Shea et al. 1993, Ortiz et al. 2002, Fuserlier and McLetchie 2004, Stehlik and Barrett 2005). For example, males of the salt grass *Distichlis spicata* grow in more saline conditions than do females (Freeman et al. 1976), and males of shadscale, *Atriplex confertifolia*, tend to predominate on dry slopes, while females are more often found on deeper and more mesic alluvial soils (Freeman et al. 1976). Spatial segregation of the sexes along resource gradients is a visual manifestation of partial niche separation by the sexes of such plants. This partial niche separation, due to environmental conditions, may result in different competitors for each sex. Historically, studies of spatial segregation have looked mainly at responses to physical or chemical resource gradients (though see Freeman and Harper 1980). Here we evaluate whether male and female plants occupying different niches have different competitors and therefore respond differently to these competitors. We also estimate the magnitude of these interactions and

express them in terms of *A. bonnevillensis* equivalents.

To evaluate responses and interactions of male and female *A. bonnevillensis*, we required a method that examines interspecific competition. Ecology abounds with such methods, ranging from manipulative approaches, such as the DeWitt replacement series (Harper 1977), to descriptive studies that express the fitness components of one species as a function of its competitors' densities (Ross and Harper 1972). Recently, Emlen et al. (2003, 2006), using an assumption of ideal-free distribution, developed the method of interaction assessment (INTASS) for assessing community-wide species interactions using simple field-derived importance values (e.g., cover). However, Rinella (personal communication) conjectured that unless microsite fertility is included, analyses of interactions could falsely suggest that interspecific facilitation as fitness components should be greater for *both* species when they are reared at more fertile sites. Only when site fertility is incorporated as a variable in the analysis can the true relationship be reliably detected. Here we take into account site fertility and use Emlen's INTASS method to examine whether or not males and females of *A. bonnevillensis* are equally influenced by their associated species.

¹Department of Biological Sciences, Wayne State University, Detroit, MI 48202.

²Western Fisheries Research Center, USGS, BRD, 6505 NE 65th St., Seattle, WA 98115.

³USDA-ARS Fort Keogh LARRL, 243 Fort Keogh Road, Miles City, MT 59301.

⁴E-mail: al8577@wayne.edu

METHODS

Our target plant, *A. bonnevillensis*, is a dioecious perennial shrub from the family Chenopodiaceae that displays spatial segregation of the sexes. It is native to the southern United States and is currently found in the deserts of Utah and Nevada. *Atriplex bonnevillensis* is capable of occupying high-saline sites on the edge of the desert playa, which is where we located our experiment. Because of high soil salinity, the ecosystem is simple, consisting of few plants, which simplifies the process of establishing and analyzing competitors. While many plant species grow on the playa, (e.g., *A. bonnevillensis*, *Halogeton glomeratus*, *Salsola kali*, *Salsola iberica*, *Eurotia lanata*, and *Chrysothamnus nauseosus*) on the edge of the playa, where saline conditions are high, species variability is very low. Our population covered an area of 4–5 ha, with only 4 consistent species: *A. bonnevillensis*, *S. kali*, *S. iberica*, and *H. glomeratus*, all chenopods. *Salsola kali*, *S. iberica*, and *H. glomeratus* are invasive weeds introduced from Eurasia. They are halophytes and do very well on the dry, salty sands of the playa. Establishing in disturbed areas, they have spread easily and quickly due to the harsh grazing of the West (Duda et al. 2003).

Our study site is located on land set aside in 1934 by the United States Department of Agriculture for the Desert Experimental Range (DER) to conduct research on the effects of grazing in the cold-desert ecosystems of the Great Basin (Hutchings and Stewart 1953, Holmgren and Hutchings 1972, Clary and Holmgren 1982, Blaisdell and Holmgren 1984). Experimental regimes of various grazing intensities and seasonality were established, in replicate, in paddocks approximately 0.80×1.61 km in size (0.5×1 mile). The DER has an annual precipitation of 22.6 cm and an average temperature range of 0.7–18.6 °C. Surrounded by mountains, a saline playa has formed in the middle of these grazed lands. This particular site is not used for grazing studies but is open to foraging cattle.

We randomly selected 19 male and 25 female *A. bonnevillensis* target plants and centered 2 circular nested quadrats (1 m² [inner] and 2.25 m² [outer]) around each plant to establish close neighbors and very close neighbors. Using modified Daubenmire (1959) cover classes, we estimated the cover of all plant

species and the bare ground in each quadrat. We also measured the height and crown diameter of each target plant, which allowed us to estimate the plant's volume as well as the cover of nontarget *A. bonnevillensis* in the same quadrat.

For purposes of the INTASS analysis (for further details see Emlen and Springman 2007), an "individual" is generally considered to be the seed or, in the case of males, some other measure of reproductive contribution that can be expressed as a seed equivalent. For females, we estimated number of seeds by averaging the number of seeds counted on 3 randomly chosen inflorescences on the target plant and then multiplying this average by the number of inflorescences. Male contributions were estimated in a similar manner; we randomly selected 4 branches with male inflorescences and measured the total length of each inflorescence. The average of these 4 lengths multiplied by the total number of inflorescences provided a measure of reproductive contribution for each target plant. To equate units of reproductive contribution for the 2 sexes, we used the average number of seeds per plant multiplied by the proportion of the sample population that was female to yield the total number of seeds in the sample. Then, as males pass on half the genes in a population, we computed the inflorescence length per seed. This method allowed us to express male reproduction in terms of seed equivalents.

Plant volume was determined using an ellipse based on measurements taken for height and crown.

Statistical Analyses

We first analyzed the data using the classical regression method (Zar 2010). We conducted simple regression analyses with the number of seeds (or seed equivalents) per plant, the plant volume, and the number of seeds (or equivalents) per unit plant volume as dependent variables, and the cover of all species in the inner and outer quadrats as independent variables. We also conducted stepwise multiple regressions using the various dependent variables and the cover classes of other species within the quadrats as independent variables. Next, we analyzed the data using INTASS. This time, however, we used the number of seeds per unit volume not as a dependent variable but rather as a measure of quadrat

TABLE 1. Applied to our data, the classical method of determining competition yields significant positive correlations ($\alpha = 0.05$) between the number of seeds per plant and the covers of *Salsola kali* and *S. iberica*, as well as the combined cover of both *Salsola* species. NS indicates nonsignificance, and "inner" and "outer" refer to the nested circular quadrats. Seeds per unit volume is an index of site fertility, and the correlations become weaker.

	Seeds per plant		Seeds per unit volume		Volume	
	Inner	Outer	Inner	Outer	Inner	Outer
<i>S. kali</i>	0.78	0.42	NS	0.49	0.43	NS
<i>S. iberica</i>	0.88	0.59	NS	NS	0.60	NS
<i>S. kali</i> + <i>S. iberica</i>	0.80	0.52	NS	0.43	0.44	NS

fertility. These fertility values were incorporated into the analysis, along with the values of plant and bare-ground cover from the corresponding smaller and larger quadrats.

RESULTS

Females

CLASSICAL ANALYSIS.—The log of the number of seeds was positively correlated with the log of plant volume ($R = 0.66$, $P < 0.001$). The number of seeds was also positively correlated with parent-plant cover values in both the inner ($R = 0.50$, $P < 0.01$) and outer ($R = 0.64$, $P < 0.001$) quadrats. This correlation simply indicates that larger plants tend to have more seeds. However, as the volume of the parent-plant increases, the number of seeds per unit volume declines ($R = 0.58$, $P < 0.001$). This decrease would seem to indicate negative feedback within a plant. There were significant correlations among plant volume, seeds, seeds per unit plant volume, and parental cover classes for both quadrat sizes, and between all these variables and bare ground. All of these relationships are expected and to some extent trivial; accordingly they are not shown. Significant positive correlations occurred between the number of seeds per plant and the cover of *S. kali* and *S. iberica*, as well as the combined cover of both *Salsola* species (Table 1). When we incorporated the seeds per volume as an index of site fertility, the correlations became weaker, affecting the second or third decimal place. No other correlations were significant.

We used the log of the number seeds on cover classes of all species in the quadrat as independent variables for stepwise multiple regression and found that *S. iberica* in the inner quadrat was the only variable entered in the equation. The slope for this variable (5.996) was significantly different from 0 ($t = 3.54$, P

< 0.002), indicating a facultative relationship. However, when parent-plant volume was included in the list of independent variables, it was the sole variable included in the regression. This indicates that the effect of other species is mediated through target-plant size.

INTASS ANALYSIS.—We scaled the coefficient of the effect of *A. bonnevillensis* in the inner quadrat (conspecific-density feedback) to -1.0 ($\beta\bar{x}$), with $\beta = -0.0199$. The coefficient for the effect of *A. bonnevillensis* in the outer quadrat was 0.0167 (Table 2; $t = 9.87$); and the magnitude of the effect, relative to conspecific feedback, was $\beta\bar{x} = 0.4931$. The positive coefficient indicates that, in general, the females do better when surrounded by other females. Bare ground in the inner quadrat also had a significant negative effect ($\beta = -0.0877$, $t = 8.35$, $P < 0.001$; $\beta\bar{x} = -0.4269$). Finally, the only other species to enter into the equation was *S. kali* in the inner quadrat ($\beta = -0.0607$, $t = 6.61$, $P < 0.001$; $\beta\bar{x} = -0.0777$). Unlike the classical analysis, INTASS indicated that *S. kali* was a competitor.

Males

CLASSICAL ANALYSIS.—As with females, we used the number of seed equivalents per plant, the number of seed equivalents per volume of the target, and the volume of the target plant as dependent variables. We found a significant correlation between the number of seed equivalents per plant and the cover of nonchenopod species in the inner quadrat ($R = 0.51$, $P < 0.03$). Similarly, there was a positive correlation between *H. glomeratus* cover in the outer quadrat and the natural log of the number of seed equivalents ($R = 0.499$, $P < 0.03$). As in the analysis of females, including the seed equivalents per unit plant volume as an estimate of site fertility weakened the correlation with nonchenopod species but did not affect the sign.

TABLE 2. INTASS correlation. The coefficient of the effect of *Atriplex bonnevillensis* in the inner quadrat (conspecific-density feedback) was scaled to -1.0 ($\beta\bar{x}$), as indicated in the first row for males and females. All other coefficients are calculated relative to this. A negative coefficient indicates a negative relationship, or competition. A positive coefficient indicates a facultative relationship. The t value indicates the significance of the relationship. Dashes indicate that the corresponding variable dropped out of the analysis either because it was highly correlated with another variable or accounted for <0.01 of the variance in fitness. In the case of female *A. bonnevillensis* in the inner quadrat, the numerical impact was scaled to -1 (i.e., fixed), so a t value is not applicable.

Sex	Quadrat	Cover	Numerical contribution to fitness		
			β	$\beta \times \text{per-individual mean of variable}$	Student's t
Female	Inner	<i>A. bonnevillensis</i>	-0.0199	-1.000	—
		<i>S. kali</i>	-0.0607	-0.078	6.61
		<i>S. iberica</i>	—	—	—
		<i>H. glomeratus</i>	—	—	—
		Bare ground	-0.0877	-0.427	8.35
	Outer	<i>A. bonnevillensis</i>	0.0167	0.493	9.87
		<i>S. kali</i>	—	—	—
		<i>S. iberica</i>	—	—	—
		<i>H. glomeratus</i>	—	—	—
		Bare ground	—	—	—
Male	Inner	<i>A. bonnevillensis</i>	-0.0198	-1.000	—
		<i>S. kali</i>	—	—	—
		<i>S. iberica</i>	—	—	—
		<i>H. glomeratus</i>	-0.0070	-0.1304	42.7
		Bare ground	—	—	—
	Outer	<i>A. bonnevillensis</i>	0.0287	0.7890	9.87
		<i>S. kali</i>	—	—	—
		<i>S. iberica</i>	—	—	—
		<i>H. glomeratus</i>	—	—	—
		Bare ground	—	—	—

INTASS ANALYSIS.—We again scaled the magnitude of the effect of male *A. bonnevillensis* in the inner quadrat equal to -1 and, as with females, found the effect of *A. bonnevillensis* individuals in the outer quadrat to be positive (Table 2; $\beta = -0.0287$, $\beta\bar{x} = 0.7887$). *Halogeton glomeratus* was the only species that significantly influenced *A. bonnevillensis* performance ($\beta = -0.0070$, $t = -42.75$, $P < 0.001$; $\beta\bar{x} = -0.1304$). No other variable, including bare ground, approached significance.

DISCUSSION

Previously, partial niche separation by the sexes of dioecious plants was demonstrated using a variety of habitat and resource gradients. Male and female plants tend to spatially segregate along such axes. This particular demonstration of partial niche separation relates to Lack's hypothesis (1947), which demonstrated that closely related species of birds could coexist provided there was niche separation along at least one axis. Like all such

partial niche separations, the niche between the sexes of dioecious plants minimizes the competition between the sexes. However, it should also bring male and female plants into contact and competition with different species. Originally, Freeman et al. (1976) argued that males and females differentially utilize at least some resources. Subsequent work (Tiedemann et al. 1987, Freeman et al. 1993a, 1993b, 1993c, Delph and Meagher 1995, Case and Ashman 2007) has confirmed that male and female reproduction utilize different amounts of plant nutrients and water (Freeman and McArthur 1982, Freeman et al. 1993a, 1993b, 1993c, Dawson and Ehleringer 1993). Thus male and female reproductive potential, and therefore reproductive success, may differ depending upon the habitat observed (Freeman et al. 1993a, 1993b, 1993c).

We found that male and female *A. bonnevillensis* have different competitors, allowing them to utilize different resources and adapt to different microsites. This also suggests that phytosociological conditions could differentially

influence male and female reproductive success, but virtually no consideration has been given to this possibility in the literature. Our work suggests that should *H. glomeratus* continue to expand, male *A. bonnevillensis* reproduction would decline, but female *A. bonnevillensis* reproductive potential would be unaffected. Although if pollen becomes limiting, reproduction would be adversely affected. Conversely, increases in the population abundance of *Salsola* species could negatively influence fruit and seed production without affecting pollen production.

Using a classical analysis, we obtained positive correlations between both species of *Salsola* and the dependent variables we measured on *A. bonnevillensis* females, whether or not we corrected for site fertility. Male reproduction was similarly categorized as facilitated by nonchenopod species in the inner quadrats and facilitated by *H. glomeratus* in the outer quadrats. These correlations are what we would expect if Rinella's conjecture on site fertility were true. Additionally, male and female plants appeared to be influenced by different species. The cover of nonchenopod species did not correlate significantly with any aspect of female size or reproduction. Similarly, the 2 species of *Salsola* (which were both significantly correlated with female size and reproduction) did not correlate with any aspect of male size or reproduction. These differences indicate differential competition of the sexes, which coincides with our hypothesis stemming from the observation that dioecious plants often exist in patterns of partial niche segregation. Our results do, however, open a new set of questions to explore, including whether differential competition is a result of or a precursor to spatial segregation.

The INTASS analysis differs from the classical analysis, yet both analyses show that different species influence male and female individuals of *A. bonnevillensis*. The classical analysis indicates that *Salsola* facilitates growth and reproduction of *A. bonnevillensis* females, while nonchenopod species and *H. glomeratus* facilitate male reproduction. INTASS suggests that *Salsola* competes with females of *A. bonnevillensis*, while *H. glomeratus* competes with males of *A. bonnevillensis*. So by either analysis, different associated species influence male and female reproduction. Thus, we demonstrated that male and female plants occupy

different niches and respond differently to competitors.

Although our use of seeds per volume per plant as a correction factor is not conventional, our approach is satisfactory considering the time and money required for alternatives. Ideally, we would have data on plant macro- and micronutrients, which would allow a more rigorous examination of site fertility. However, from a practical standpoint, if land managers had to resort to soil analyses for every quadrat, the expense would preclude any practical assessments of interspecific interactions and thus fail to inform land managers about the importance of interspecific interactions.

In conclusion, both types of analyses show that different species influence male and female *A. bonnevillensis* reproduction, as theory would predict. The results of the classical analysis, however, conflict with standard ecological wisdom that indicates that *Salsola* and *H. glomeratus* are competitors and not facilitative towards perennial *Atriplex* species. The INTASS analysis was in accordance with standard wisdom.

LITERATURE CITED

- BARRETT, S.C.H., AND J.D. THOMSON. 1982. Spatial pattern, floral sex ratios, and fecundity in dioecious *Aralia nudicaulis* (Araliaceae). *Canadian Journal of Botany* 60:1662–1670.
- BLAISDELL, J.P., AND R.C. HOLMGREN. 1984. Managing intermountain rangelands: salt-desert shrub ranges. General Technical Report 163, USDA Forest Service, Intermountain Forest and Range Experiment Station. 52 pp.
- CASE, A.L., AND T.-L. ASHMAN. 2007. An experimental test of the effects of resource and sex ratio on maternal fitness and phenotypic selection in gynodioecious *Fragaria virginiana*. *Evolution* 61:1900–1911.
- CLARY, W.P., AND R.C. HOLMGREN. 1982. Desert experimental range: establishment and contribution. *Rangelands* 4:261–264.
- COX, P.A. 1981. Niche partitioning between sexes of dioecious plants. *American Naturalist* 117:295–307.
- DAUBENMIRE, R. 1959. A canopy-coverage method of vegetational analysis. *Northwest Science* 33:43–64.
- DAVEY, A.J., AND C.M. GIBSON. 1917. Note on the distribution of sexes in *Myrica gale*. *New Phytologist* 16: 147–150.
- DAWSON, T.E., AND L.C. BLISS. 1989. Patterns of water use and the tissue water relations in the dioecious shrub, *Salix arctica*: the physiological basis for habitat partitioning between the sexes. *Oecologia* 79:332–343.
- DAWSON, T.E., AND J.R. EHLERINGER. 1993. Gender-specific physiology, carbon isotope discrimination, and habitat distribution in boxelder, *Acer negundo*. *Ecology* 74: 798–815.

- DELPH, L., AND T. MEAGHER. 1995. Sexual dimorphism masks life history trade-offs in the dioecious plant *Silene latifolia*. *Ecology* 76:775–785.
- DUDA, J.J., D.C. FREEMAN, J.M. EMLÉN, J. BELNAP, S.G. KITCHEN, J.C. ZAK, E. SOBEK, M. TRACY, AND J. MONTANTE. 2003. Differences in native soil ecology associated with invasion of the exotic annual chenopod, *Halogeton glomeratus*. *Biology and Fertility of Soils* 38:72–77.
- EMLÉN, J.M., J.J. DUDA, M.D. KIRCHHOFF, AND D.C. FREEMAN. 2006. Interaction assessment: a modeling tool for predicting population dynamics from field data. *Ecological Modeling* 192:557–570.
- EMLÉN, J.M., D.C. FREEMAN, M.D. KIRCHHOFF, C.L. ALADOS, J. ESCÓS, AND J.J. DUDA. 2003. Fitting population models from field data. *Ecological Modeling* 162:119–143.
- EMLÉN, J.M., AND K.R. SPRINGMAN. 2007. Developing methods to assess and predict the population level effects of environmental contaminants. *Integrated Environmental Assessment and Management* 3:157–165.
- FREEMAN, D.C., AND K.T. HARPER. 1980. A relationship between competition and sex ratio of shadscale. *Journal of Range Management* 33:236–237.
- FREEMAN, D.C., L.G. KLIKOFF, AND K.T. HARPER. 1976. Differential resource utilization by the sexes of dioecious plants. *Science* 193:597–599.
- FREEMAN, D.C., AND E.D. MCARTHUR. 1982. A comparison of water stress between males and females of four species of desert shrubs. *Forest Science* 28:304–308.
- FREEMAN, D.C., E.D. MCARTHUR, S.C. SANDERSON, AND A.R. TIEDEMANN. 1993a. The influence of topography on male and female fitness components of *Atriplex canescens*. *Oecologia* 93:538–547.
- . 1993b. Influence of topography on cumulative pollen flow of fourwing saltbush. United States Department of Agriculture, Forest Service, Intermountain Research Station.
- . 1993c. The influence of topography on pollen flow in *Atriplex canescens*. USDA Forest Service Intermountain Research Station, Research Note INT-413. 9 pp.
- FOX, J.F., AND A.T. HARRISON. 1981. Habitat assortment of sexes and water balance in a dioecious grass. *Oecologia* 49:233–235.
- FUSELIER, L., AND D. MCLETHIE. 2004. Microhabitat and sex distribution in *Marchantia inflexa*, a dioecious liverwort. *Bryologist* 107:345–356.
- GRANT, M.C., AND J.B. MITTON. 1979. Elevation gradients in adult sex ratios and sexual differentiation in vegetative growth rates of *Populus tremuloides* Michx. *Evolution* 33:914–918.
- GREGG, K.B. 1975. The effect of light intensity on sex expression in species of *Cynoches* and *Catasetum* (Orchidaceae). *Selbyana* 1:101–113.
- HARPER, J.L. 1977. Population biology of plants. Academic Press. 892 pp.
- HOLMGREN, R.C., AND S.S. HUTCHINGS. 1972. Salt desert shrub response to grazing use. Wildland shrubs: their biology and utilization. Pages 153–165 in Technical Report INT-1, USDA Forest Service, Intermountain Forest and Range Experiment Station, Ogden, UT.
- HUTCHINGS, S.S., AND G. STEWART. 1953. Increasing forage yields and sheep production on intermountain winter ranges. U.S. Department of Agriculture Circular 925. 63 pp.
- LACK, D. 1947. Darwin's finches. *American Naturalist* 81:462–467.
- LOVETT DOUST, J., G. O'BRIAN, AND L. LOVETT DOUST. 1987. Effect of density on secondary sex characteristics and sex ratio in *Silene alba* (Caryophyllaceae). *American Journal of Botany* 74:40–46.
- ORTIZ, P.L., M. ARISTA, AND S. TALAVERA. 2002. Sex ratio and reproductive effort in the dioecious *Juniperus communis* subsp. *alpina* (Suter) Celak (Cupressaceae) along an altitudinal gradient. *Annals of Botany* 89:205–211.
- ROSS, M.A., AND J.L. HARPER. 1972. Occupation of biological space during seedling establishment. *Journal of Ecology* 60:77–88.
- SHEA, M.M., P.M. DIXON, AND R.R. SHARITZ. 1993. Size differences, sex ratio, and spatial distribution of male and female water tupelo, *Nyssa aquatica* (Nyssaceae). *American Journal of Botany* 80:26–30.
- STEHLIK, I., AND S. BARRETT. 2005. Mechanisms governing sex-ratio variations in dioecious *Rumex nivals*. *Evolution* 59:814–825.
- TIEDEMANN, A.R., E.D. MCARTHUR, AND D.C. FREEMAN. 1987. Variation in physiological metabolites and chlorophyll in sexual phenotypes of 'Rincon' fourwing saltbush. *Journal of Range Management* 40:151–155.
- VITALE, J.J., AND D.C. FREEMAN. 1986. Partial niche separation in *Spinacia oleracea* L.: an examination of reproductive allocation. *Evolution* 40:426–430.
- WADE, K.M. 1981. Experimental studies on the distribution of the sexes of *Mercurialis perennis* L. III. Transplanted populations under light screens. *New Phytologist* 87:447–455.
- WASER, N.M. 1984. Sex ratio variation in populations of a dioecious desert perennial, *Simmondsia chinensis*. *Oikos* 42:343–348.
- ZAR, J.H. 2010. Biostatistical analysis. 5th edition. Pearson Prentice-Hall, Upper Saddle River, NJ. 944 pp.

Received 8 September 2008

Accepted 28 May 2009