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Ginsenoside content and variation among and within American ginseng (*Panax quinquefolius* L.) populations

Erin M. Schlag, Marla S. McIntosh *

Department of Natural Resource Sciences and Landscape Architecture, University of Maryland, 2102 Plant Sciences Building #36, College Park, MD 20742, United States

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Abstract

The contents of five ginsenosides (Rg1, Re, Rb1, Rc and Rd) were measured in American ginseng roots collected from 10 populations grown in Maryland. Ginsenoside contents and compositions varied significantly among populations and protopanaxatriol (Rg1 and Re) ginsenosides were inversely correlated within root samples and among populations. The most abundant ginsenoside within a root and by population was either Rg1 or Re, followed by Rb1. Ginseng populations surveyed grouped into two chemotypes based on the relative compositions of Rg1 and Re. Four populations, including the control population in which plants were grown from TN and WI seed sources, contained roots with the recognized chemotype for American ginseng of low Rg1 composition relative to Re. The remaining 6 populations possessed roots with a distinctive chemotype of high relative Rg1 to Re compositions. Chemotype did not vary by production type (wild versus cultivated) and roots within a population rarely exhibited chemotypes different from the overall population chemotype. These results provide support for recent evidence that relative Rg1 to Re ginsenoside contents in American ginseng roots vary by region and that these differences are likely influenced more by genotype than environmental factors. Because the physiological and medicinal effects of different ginsenosides differ and can even be oppositional, our findings indicate the need for fingerprinting ginseng samples for regulation and recommended usage. Also, the High Rg1/Low Re chemotype discovered in MD could potentially be used therapeutically for coronary health based on recent evidence of the positive effects of Rg1 on vascular growth.

Keywords: *Panax quinquefolius*; Araliaceae; American ginseng; Saponins; Ginsenosides; Chemical variation; Rg1; Re

1. Introduction

American ginseng (*Panax quinquefolius* L.) and Asian ginseng (*Panax ginseng*) roots are among the most widely-used traditional Chinese medicines. Recently, ginseng roots and their extracts have also become popular in the US and Europe as dietary health supplements and additives to foods and beverages. Although plants of both the Asian and American species are valued for their adaptogenic properties, these species have different uses according to traditional Chinese herbal guides. Asian ginseng is considered to be stimulating and invigorate yang, whereas American ginseng is considered to be calming and nourishing yin (Dharmananda, 2002). The pharmacological effects of ginseng roots have been attributed primarily to ginsenosides, which are triterpenoid saponin glycosides (dammarene-type saponins) (Fig. 1). More than 27 putative ginsenosides have been isolated from ginseng roots and are classified into two main groups: the glycosides of 20(*S*)-protopanaxadiol (20[*S*]-dammar-24-ene-3β, 12β, 20-triol) (Rb1, Rb2, Rc, Rd, Rg3 and Rh2, see Fig. 1) and those of 20(*S*)-protopanaxatriol (6α-hydroxy-20[*S*]-protopanaxadiol) (Re, Rf, Rg1, Rg2, Rh1 and R1, see Fig. 1) (Attele et al., 1999; Awang, 2000; Shibata, 2001). The main ginsenosides isolated from American ginseng (Rb1, Rc, Rd, Re and Rg1) typically account for greater than 70% of the total ginsenosides (Court et al., 1996b; Wills et al.,

* Corresponding author. Tel.: +1 301 405 1323; fax: +1 301 314 9308.
E-mail address: mmcintos@umd.edu (M.S. McIntosh).

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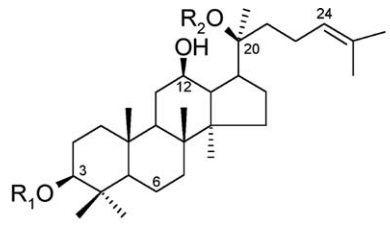
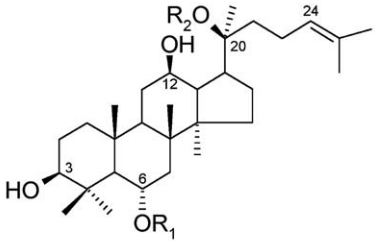
A



Ginsenoside	R_1	R_2
Rb1	-Glc^2-Glc	-Glc^6-Glc
Rb2	-Glc^2-Glc	-Glc^6-Ara(p)
Rc	-Glc^2-Glc	-Glc^6-Ara(f)
Rd	-Glc^2-Glc	-Glc
Rg3	-Glc^2-Glc	-H
Rh2	-Glc	-H

B



Ginsenoside	R_1	R_2
Re	-Glc^2-Rha	-Glc
Rf	-Glc^2-Glc	-H
Rg1	-Glc	-Glc
Rg2	-Glc^2-Rha	-H
Rh1	-H	-H

Fig. 1. Chemical structures of two major groups of ginsenosides: (A) 20(S) protopanaxadiols and (B) 20(S) protopanaxatriols. Glc, glucose; Ara(p), arabinose in pyranose form; Ara(f), arabinose in furanose form; Rha, rhamnose; H, hydrogen. Content of figure is adapted from Attele et al. (1999) and Shibata (2001).

2002; Assinewe et al., 2003). Factors found to affect ginsenoside contents of American ginseng roots include age (Court et al., 1996a; Smith et al., 1996; Wills et al., 2002; Lim et al., 2005), root dry weight (Wills et al., 2002), soil fertility (Li and Mazza, 1999), light (Fournier et al., 2003), and population/location (Li et al., 1996; Assinewe et al., 2003; Lim et al., 2005). However, the relative rankings of the main ginsenoside constituents in cultivated American ginseng root samples have been consistent across studies with the approximate profile, Rb1 > Re > Rg1 = Rc > Rd (Court et al., 1996b; Wills et al., 2002; Assinewe et al., 2003; Wang et al., 2005).

Recent clinical and laboratory studies have verified that roots of American and Asian ginseng have contradictory effects on the vascular system (Sengupta et al., 2004) and acute glycemia (Sievenpiper et al., 2004). The different bioactivities have been experimentally linked to ginsenoside fingerprint; variation attributed to differences between species, geographical origin and/or species dependent extraction methods (Sengupta et al., 2004; Sievenpiper et al., 2004). Sengupta et al. (2004) reported that Asian ginseng had a high Rg1:Rb1 ginsenoside ratio and Rg1 was shown to promote wound healing. Conversely, American ginseng had a low Rg1:Rb1 ratio and Rb1 was shown to inhibit tumor growth. The American ginseng roots in this study (Sengupta et al., 2004) produced uniform ginsenoside profiles considered characteristic of American ginseng. However, if the differences in ginsenoside profiles between species are genetically controlled, then it is likely that the profiles would also vary within the *P. quinquefolius* species because wild American ginseng populations are often geographically isolated (Cruse-Sanders and Hamrick, 2004; Grubbs and Case, 2004). Although typically American ginseng is thought to have Rg1:Rb1 ratios of less than 1.0 (~0.15), wild roots have been reported to contain much higher levels of Rg1 (Chuang et al., 1995; Assinewe et al., 2003; Lim et al., 2005). Sievenpiper et al. (2004) reported that American ginseng from two sources differed both in Rg1 content and in its effect on glycemic indices.

Over the last three centuries, the high value and a strong export market have led to overharvesting of wild American ginseng. Although American ginseng has been cultivated since the 1880s, its market export value is considerably less than the value of wild ginseng (Beyfuss, 1999; Chamberlain and Predny, 2005). The combined effects of overharvesting, deforestation, and land development threaten the number and size of wild American ginseng populations. *P. quinquefolius* L. is listed in CITES Appendix II and its harvest and commerce are regulated by the US Fish and Wildlife Service. Although ecological and biological assessments of American ginseng populations are needed to effectively develop policies for the long term sustainability of native American ginseng, there is an information gap attributed to inadequate funding (Robbins, 2000). In addition, studies of wild ginseng populations, especially those involving roots, are limited because population sizes are typically small (McGraw et al., 2003), ginseng collectors tend not to reveal collection sites, and root sampling is destructive and poses a threat to protected native populations.

Assinewe et al. (2003) provided the first comprehensive report of ginsenoside contents of roots collected from wild populations. Roots from 10 populations growing in the US and Canada, in the northern portion of the native ginseng range, were analyzed. Like cultivated samples, Rb1 contents in these Northern wild populations were greater than the contents of either Rg1 or Re (Court et al., 1996b; Wills et al., 2002; Wang et al., 2005) and variation of the content of protopanaxatriol ginsenosides (Rg1 and Re) among roots was less than found for panaxadiol ginsenosides (Rb1, Rc and Rd) (Li et al., 1996). However, Rg1 content was higher than previously reported, such that the Rg1 and Re contents were not significantly different from each other. Lim et al. (2005) analyzed roots collected from eight wild American ginseng populations found in the Catskill

region of NY and reported that the mean ginsenoside content of these NY wild populations was also highest for Rb1. However, the Rg1 and Re contents were found to vary among populations (and in some cases, among roots within a population) and Rg1 and Re contents within a plant were inversely related. This study suggested that Re content was influenced by genotype, Rb1, Rc, and Rb2 contents were influenced by location, and Rg1 and Rd contents were influenced by both (Lim et al., 2005).

Our study presents individual and total ginsenoside contents and compositions of wild and cultivated American ginseng populations grown in the Appalachian and Piedmont physiographic regions of MD. Although these physiographical regions in MD are located at the center of American ginseng's native range, populations from these regions have not previously been studied. This report is the first to describe the phytochemical properties of American ginseng roots grown in MD in cultivated and wild populations purportedly derived from MD seed sources and to compare their phytochemistry to roots also grown in MD from TN and WI seed sources. TN and WI are among the top states for wild and cultivated American ginseng production, respectively (Chamberlain and Predny, 2005).

2. Results and discussion

Contents of the major ginsenosides, Rg1, Rb1, Re, Rc, and Rd were quantified using HPLC for 44 American ginseng roots from 10 populations grown in MD. The populations differed in production system (wild vs. cultivated) and seed source (Table 1). Except for roots from population F2EX, all cultivated samples were grown from seed that purportedly had been collected during the past few decades from wild Maryland populations. For the 18 wild roots in our study, total ginsenoside contents ranged from 0.85% (w/w) to 5.78% with an overall mean ± SD of 2.50 ± 1.28% and a median of 1.93%. For the 26 cultivated roots, the range was slightly less (1.04–4.07%) but the overall mean ± SD of 2.25 ± 0.85% and median (1.96%) was not different from that of the wild populations. The overall mean total ginsenoside contents of these MD grown populations was similar to NY wild populations (2.7%) (Lim et al., 2005) but relatively low compared to reported means of four year old cultivated American ginseng (2–6%) (Court et al., 1996a; Smith et al., 1996; Wills et al., 2002). The overall mean total ginsenoside reported for wild ginseng populations in the northern US and Canada was two times higher (5.78%) (Assinewe et al., 2003) than the mean for wild MD populations. As in the MD populations, total ginsenosides of wild roots collected in the northern US and Canada were highly variable and individual root contents ranged from 1% to 15% w/w (Assinewe et al., 2003).

Variability for total ginsenosides is inherently large because it includes the variability associated with each ginsenoside. Unexplained variation due to the spatial variability of the forest environment and variable plant ages, in conjunction with limited sampling sizes, present significant obstacles to obtaining sufficient statistical power to detect significant differences between means. Despite the large unexplained variation and small sample sizes, median total ginsenosides were significantly different among populations ($\chi^2 = 21.3$, $p < 0.05$) (Table 2). The most probable causes of differences among populations include genotype, environment, and/or plant age. Plant age affects root ginsenoside content in cultivated and wild populations (Court et al., 1996a; Smith et al., 1996; Wills et al., 2002; Lim et al., 2005) and plants younger than four years of age are considered unsuitable for harvest because of low ginsenoside contents (Court et al., 1996b). In our study, total ginsenosides of roots that were less than five years old were uniformly low in total ginsenosides, while there was no apparent relationship between age and total ginsenoside contents for roots 5 years or older (Fig. 2). Thus, for populations F2EX and P1WD, consisting of plants that were three and four years old, age may have contributed to

Table 1
Population code, location grown, putative seed source, sample size and median root age and interquartile range of American ginseng (*Panax quinquefolius*) roots collected in wild and cultivated populations in Maryland

Population	Location grown	Putative seed source	Collection year	Sample size (*n*)	Median age[a]
Cultivated					
F2EX	Allegheny County	TN, WI[b]	2002–2003	7	4 (4–4)
F1MD	Allegheny County	Allegany County	2003	2	6 (4–8)
F2MD	Allegheny County	Garrett County	2002–2003	3	6 (6–8)
F3MD	Garrett County	Garrett County	2002–2003	12	8 (6–8)
F4MD	Frederick County	Garrett County	2003	2	6 (6–6)
Wild					
P1WD	Garrett County	Garrett County	2003	2	4 (4–4)
P3WD	Garrett County	Garrett County	2002–2003	6	8 (8–10)
P5WD	Washington County	Washington County	2003	3	6 (6–6)
P7WD	Washington County	Washington County	2002–2003	4	8 (6–10)
P9WD	Allegany County	Allegany County	2003	3	10 (6–10)

[a] Root age was estimated by counting the number of bud scars within the neck of the root.
[b] Plants cultivated from seed purchased from commercial plots in Tennessee (TN) and Wisconsin (WI).

Table 2
χ^2-Values for Kruskal–Wallis tests for significant differences in root ginsenoside contents and compositions among American ginseng (*Panax quinquefolius*) populations grown in Maryland

		Ginsenoside content (% w/w)					
		χ^2-value					
Source of variation	df	Rg1	Re	Rb1	Rc	Rd	Total
Population	9	30.5‡	24.6†	21.0*	19.3*	12.7	21.3*
		Ginsenoside composition (% total ginsenoside content)					
		χ^2-value					
Source of variation	df	Rg1	Re	Rb1	Rc	Rd	
Population	9	31.8‡	27.7‡	20.2*	17.1*	28.8‡	

*, †, ‡ χ^2-values are significant at the 0.05, 0.01 and 0.001 levels, respectively.

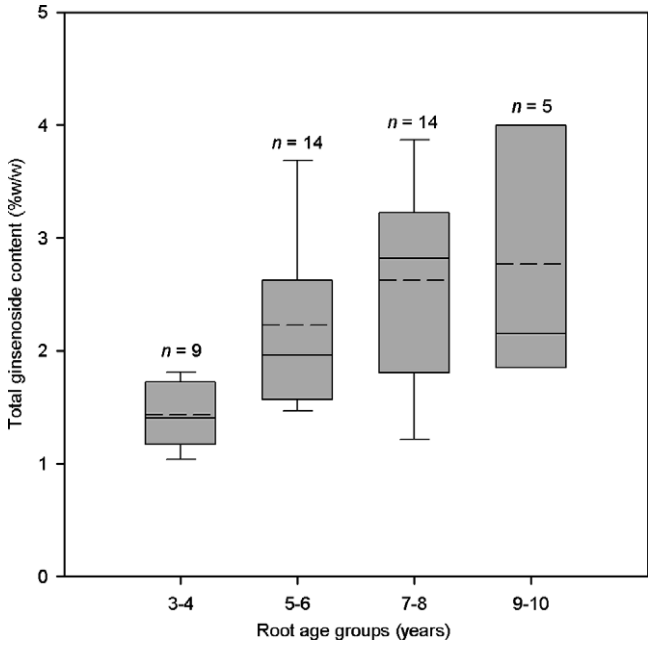


Fig. 2. Boxplot of total American ginseng (*Panax quinquefolius*) root ginsenoside content (% w/w) by age group.

lower total ginsenoside contents (Tables 1 and 3). In wild NY populations, only Rb1, Rc, Rd and total ginsenoside contents were affected by root age (Lim et al., 2005), thus root age may be of particular importance when high levels of panaxadiol ginsenosides or total ginsenoside are desired for therapeutic purposes. Of the five ginsenosides measured in this study, Rb1 was the most highly correlated with total ginsenoside content ($r = 0.96$, $p < 0.001$) and therefore, in situations in which resources are limited, measurement of Rb1 alone appears to be indicative of the total ginsenoside content.

Wild ginseng is easily distinguished from cultivated ginseng by the root form and color. Asian buyers consider wild ginseng to be more potent than cultivated ginseng (Robbins, 2000) and root value is highly dependent on its morphology. In 2002, the average US Food and Agricultural Service Value for exported American ginseng was \$39/kg for cultivated roots and \$139/kg for wild roots (Blumenthal, 2003). Although there is a large difference in market value between wild and cultivated ginseng, we did not find a significant difference between wild and cultivated plants for mean total ginsenoside content (Wilcoxon

Table 3
Median ginsenoside contents and interquartile ranges of the median for cultivated and wild populations of American ginseng (*P. quinquefolius*) in Maryland

Population	n[b]	Ginsenoside content[a] (% w/w)					
		Rg1	Re	Rb1	Rc	Rd	Total
F2EX	7	0.10 (0.07–1.41)	0.77 (0.72–0.83)	0.43 (0.29–0.50)	0.14 (0.11–0.15)	0.10 (0.06–0.11)	1.54 (1.26–1.81)
F1MD	2	0.15 (0.12–0.18)	1.13 (0.62–1.63)	0.99 (0.39–1.69)	0.17 (0.15–0.18)	0.08 (0.07–0.09)	2.50 (1.34–3.66)
P7WD	4	0.23 (0.09–0.31)	0.99 (0.92–1.72)*	1.15 (0.67–1.49)*	0.28 (0.17–0.39)*	0.06 (0.05–0.09)	2.74 (1.95–3.92)*
P5WD	3	0.53 (0.47–0.91)*	1.23 (1.14–1.94)*	1.55 (1.31–2.82)*	0.31 (0.22–0.40)*	0.10 (0.08–0.15)	3.72 (3.65–5.78)*
P9WD	3	0.76 (0.19–0.92)*	0.15 (0.13–1.10)	0.51 (0.51–0.56)	0.23 (0.16–0.33)*	0.05 (0.05–0.07)	1.85 (1.85–2.00)
P1WD	2	0.65 (0.51–0.78)+	0.15 (0.13–0.17)+	0.43 (0.26–0.59)	0.15 (0.14–0.16)	0.06 (0.04–0.08)	1.43 (1.08–1.78)
P3WD	6	0.78 (0.54–1.16)*	0.18 (0.03–0.30)*	0.46 (0.36–0.77)	0.24 (0.18–0.30)*	0.08 (0.06–0.10)	1.67 (1.39–2.56)
F4MD	2	0.99 (0.99–1.00)+	0.11 (0.05–0.16)+	0.58 (0.55–0.60)	0.22 (0.19–0.24)+	0.06 (0.05–0.06)	1.95 (1.95–1.95)
F2MD	3	1.03 (0.71–1.44)*	0.04 (0.00–0.12)*	0.49 (0.37–0.81)	0.16 (0.12–0.24)	0.05 (0.04–0.10)	1.56 (1.45–2.75)
F3MD	12	1.31 (0.68–1.79)*	0.06 (0.00–0.64)*	0.89 (0.63–1.22)*	0.21 (0.17–0.27)*	0.10 (0.07–0.12)	2.97 (2.13–3.25)*

*, + Medians significantly different from F2EX at $p < 0.05$, 0.06 levels, respectively, according to multiple Wilcoxon rank sum tests.
[a] Samples were tested in duplicate.
[b] Number of root samples tested in each population.

$Z = 0.56$, $p = 0.57$), nor did Assinewe et al. (2003). However, Lim et al. (2005) determined that when plants from the same population were grown in different cultural conditions and age was controlled, total ginsenoside content was greater in roots cultivated under wild-simulated conditions than under the more intensive woods-cultivated conditions. Although the relationship between intensity of production and total ginsenoside content remains unclear, the Asian market preference for wild ginseng and its perceived superior potency do not appear to be strongly related to total ginsenoside content. Conversely, total ginsenoside content is considered a standard measure of potency for ginseng products in the US. Manufacturers in the US often claim that their products are standardized and list the total ginsenoside content on the product label, although, without any FDA oversight, these claims may be inaccurate (Hall et al., 2001).

Traditional Chinese medicine has long recognized quality differences between ginseng species that are unrelated to total ginsenoside content (American Botanical Council, 2002). Recently, published studies have demonstrated that differences in individual ginsenoside contents between Asian and American ginseng are associated with opposite biological effects (Sengupta et al., 2004; Sievenpiper et al., 2004). This validation of the Asian perspective also suggests that ginsenoside composition may be as important as total ginsenoside content for determining recommended dosages for various therapeutic uses. Median contents and compositions of the major ginsenosides and their 25–75th interquartile ranges for wild and cultivated populations represent initial reference values for MD American ginseng (Tables 3 and 4). Medians rather than means were used as a measure of central tendency for individual ginsenoside contents and compositions because these distributions were not normal and the variances were unequal. For all of the major ginsenosides, median contents and/or composition differed significantly among populations ($p < 0.05$) (Table 2). Median ginsenoside contents and compositions of each population were compared to the F2EX population, which was considered the control because it was grown from TN and WI (non-MD) seed sources. All Maryland populations, except for F1MD, had at least one ginsenoside that was significantly different from F2EX ($p < 0.05$ or $p < 0.06$ for populations with $n = 2$) (Tables 3 and 4). Populations F3MD, P5WD and P7WD had the highest Rb1 contents and thus the highest total ginsenoside contents (Table 3), which is likely the combined effect of age

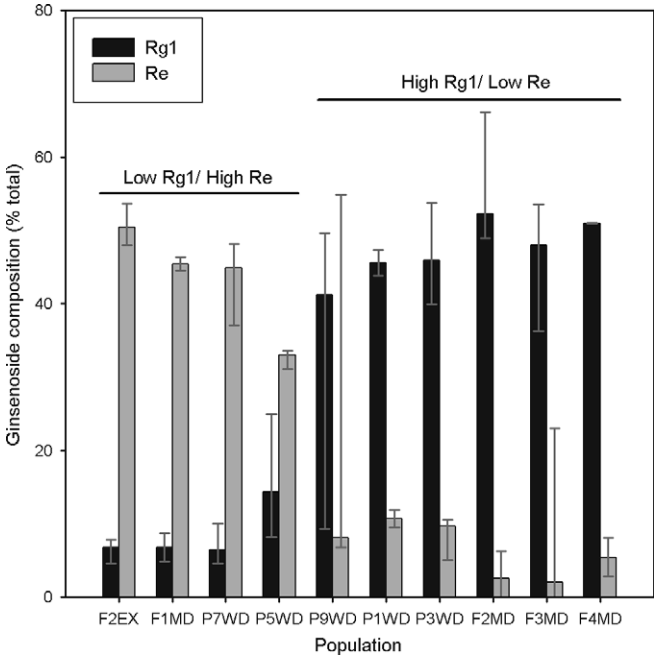


Fig. 3. Chemotypic grouping of 10 populations of American ginseng (*Panax quinquefolius*) according to median ginsenoside composition. Populations with median Rg1 <20% and median Re >30% are characterized as having a Low Rg1/High Re chemotype characteristic of American ginseng. Populations with median Rg1 >30% and median Re <20% are characterized as having a High Rg1/Low Re chemotype.

Table 4
Median ginsenoside compositions and interquartile ranges of the median for cultivated and wild populations of American ginseng (*P. quinquefolius*) in Maryland

Population	n[b]	Ginsenoside composition[a] (% total ginsenoside content)				
		Rg1	Re	Rb1	Rc	Rd
F2EX	7	7 (5–8)	50 (48–54)	28 (24–29)	9 (7–10)	6 (5–7)
F1MD	2	7 (5–9)	45 (45–46)	36 (29–43)	8 (5–11)	4 (2–5)
P7WD	4	6 (5–10)	45 (37–48)	35 (35–42)*	9 (8–11)	3 (2–3)*
P5WD	3	14 (8–25)	33 (31–34)*	42 (36–49)*	7 (6–8)	3 (2–3)*
P9WD	3	42 (9–50)*	8 (7–55)	27 (25–30)	12 (8–18)	3 (2–4)*
P1WD	2	46 (44–47)+	11 (9–12)+	29 (24–33)	11 (9–13)	4 (4–5)
P3WD	6	46 (40–54)*	10 (5–11)*	28 (27–29)	14 (13–15)+	5 (4–5)
F4MD	2	51 (51–51)+	5 (3–8)+	30 (28–31)	11 (10–12)	3 (3–3)+
F2MD	3	52 (49–66)*	3 (0–6)*	29 (24–34)	9 (7–11)	4 (3–4)*
F3MD	12	48 (36–54)*	2 (0–23)*	31 (29–37)*	8 (7–9)	4 (3–4)*

*, + Medians significantly different from F2EX at $p < 0.05$, 0.06 levels, respectively, according to multiple Wilcoxon rank sum tests.
[a] Samples were tested in duplicate.
[b] Number of root samples tested in each population.

and location (Li et al., 1996; Lim et al., 2005). However, as the median age was high relative to the control in these populations and locations variable (regionally and by production type), age may have been more of a factor than location (Table 1 and Fig. 2). The significant differences among American ginseng populations derived from variable seed sources provide additional evidence that ginsenoside profiles differ due to ecotypic variation within species as well as speciographic variation.

Differences among populations for Rb1, Rc, Rd and total ginsenoside contents and/or compositions were significant ($p < 0.05$) but markedly less than among populations for Rg1 ($p < 0.001$) and Re ($p < 0.01$) contents (Table 2). Until recently, variability in Rg1 contents had only been reported on rare occasion for wild American ginseng roots of unknown origin (Chuang et al., 1995; Awang, 2000) and variability in Re contents had not been reported at all. The median compositions of both Rg1 and Re in populations F2MD, F3MD, F4MD, P1WD and P3WD were significantly different ($p < 0.05$ or 0.06) from the control population (Table 4) and root Rg1 and Re ginsenoside contents were negatively correlated ($r = -0.70$, $p < 0.001$). These populations exhibited a significantly lower median Re composition and a significantly higher median Rg1 composition than the control (Table 4). Whereas Assinewe et al. (2003) reported approximately equal contents of Rg1 and Re and no significant difference in content of either ginsenoside among populations, we found that populations exhibited one of two opposing chemotypes; the recognized chemotype for American ginseng: low Rg1 relative to Re or a distinctive high Rg1 relative to Re chemotype. We observed a clear separation of populations by chemotype, with six populations that exhibited median Rg1 compositions >30% and Re <20% (High Rg1/Low Re chemotype) and four populations (including the control population) that exhibited median Rg1 compositions <20% and Re >30% (Low Rg1/High Re chemotype) (Fig. 3). These results are similar to those reported by Lim et al. (2005), who found that Rg1 and Re were inversely related within and among wild populations from NY.

Our study provides further evidence of wild American ginseng populations from the Eastern US that exhibit protopanaxatriol chemotypes with high Rg1 (~45%) and low Re (~10%) root ginsenoside compositions that are distinctive from chemotypes reported for commercial ginseng as well as from wild ginseng from the Northern US and Canada (Table 5). While mean Rb1, Rc and Rd compositions of MD grown populations were similar to profiles reported previously for American ginseng, the relative percentage of Rg1 was substantially higher and the relative percentage of Re was substantially lower in six of the 10 populations included in this study (Table 5). In contrast to Lim et al. (2005), we found less evidence of variation in root chemotype within populations. The majority of roots (93%) exhibited one of the two chemotypes observed at the population level: High Rg1/Low Re chemotype (Rg1 composition >30%, Re <20%) or the Low Rg1/High Re chemotype (Rg1 composition <20%, Re >30%) and the average Rg1 and Re compositions differed by a factor of six between chemotypes (see Fig. 4 for representative chromatograms). Three roots were classified as intermediate, having high Re (>30%), but intermediate Rg1 composition (20–30%). Only five out of 44 roots were not in agreement with the population chemotype, either because they were intermediate or opposing chemotypes (Table 6), suggesting that chemotype in MD is relatively similar within populations, but distinct among populations. We should note that not all roots purportedly grown from Maryland seed exhibited the High Rg1/Low Re chemotype. Two wild Maryland populations (P5WD and P9WD) and one cultivated population (F1MD) exhibited the Low Rg1/High Re chemotype associated with roots cultivated from TN and WI seed sources (F2EX) (Table 6 and Fig. 3). In addition, one root sampled each from F3MD and P9WD populations exhibited the Low Rg1/High Re chemotype (Table 6).

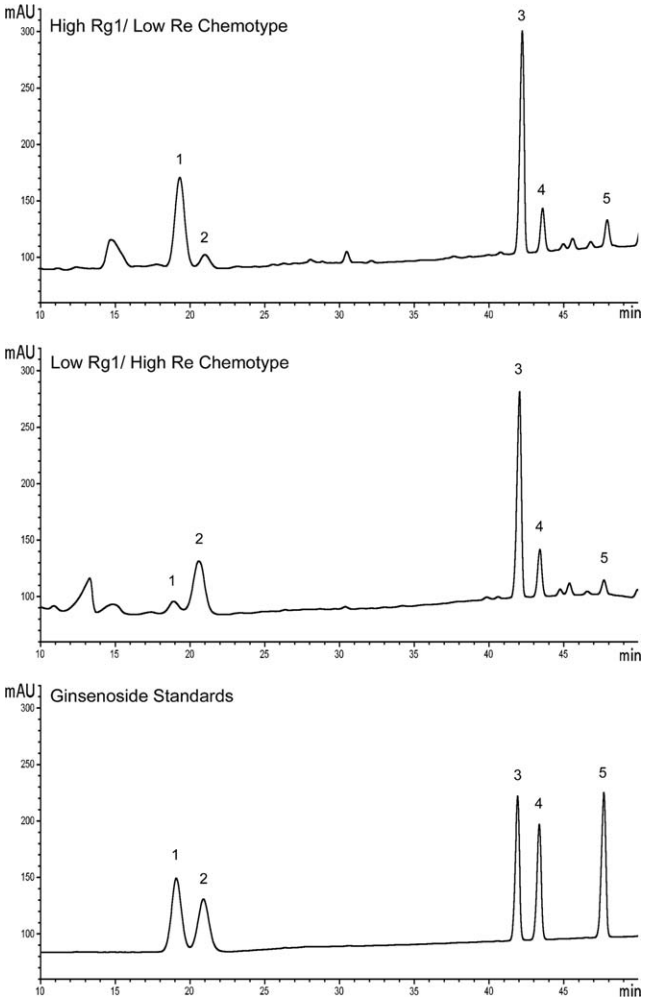


Fig. 4. Representative HPLC ginsenoside chromatograms observed in American ginseng (*Panax quinquefolius*) roots collected in Maryland and ginsenoside standards: peak 1 (Rg1), peak 2 (Re), peak 3 (Rb1), peak 4 (Rc) and peak 5 (Rd). Two chemotypes: High Rg1/Low Re and Low Rg1/High Re are represented as well as standards for authentication.

Table 5
Mean ginsenoside composition of roots in cultivated and wild American ginseng populations (*Panax quinquefolius*) in Maryland (this study) and from published results for five previous studies in the US, Canada and Australia

Chemotype[a]	Reference	Ginsenoside % w/w						Production	Age (yrs)	*n*
		% Total								
		Total	Rg1	Re	Rb1	Rc	Rd			
Low Rg1/High Re	Court et al. (1996b)[b]	4.3	4	35	45	6	10	Cultivated	4	8 (2)
Low Rg1/High Re	Li et al. (1996)	3.0	6	37	41	6	10	Cultivated	4	54 (9)
Low Rg1/High Re	Wills et al. (2002)	ND	8	40	45	3	4	Cultivated	4	10 (2)
Low Rg1/High Re	Assinewe et al. (2003)	4.9	5	36	39	7	10	Cultivated	4	12 (1)
Low Rg1/High Re	Lim et al. (2005)[c]	2.7	6	47	36	8	3	Wild	4–10	6 (1)
Low Rg1/High Re	This study[d]	2.5	9	45	33	9	4	Both	4–10	16 (4)
Intermediate	Assinewe et al. (2003)	5.8	16	24	48	7	5	Wild	ND	38 (10)
High Rg1/Low Re	Lim et al. (2005)[e]	2.5	40	13	38	7	2	Wild	4–10	7 (1)
High Rg1/Low Re	This study[f]	2.3	45	11	30	10	4	Both	4–10	28 (6)

Mean total ginsenoside content (% w/w) and ginsenoside composition (% total) were approximated from published results from one or multiple populations or field sites (also reported as gardens or farms). Root age (years), number of samples (*n*) and number of populations or field sites (in parentheses) are provided for comparison.

[a] Chemotype was based on mean Rg1 and Re ginsenoside composition: High Rg1/Low Re (Rg1 > 30%; Re < 20%), Low Rg1/High Re (Rg1 < 20%; Re > 30%) and Intermediate (15% < Rg1, Re < 30%).

[b] Total ginsenoside based on sum of values for Rg1, Re, Rb1, Rc and Rd.

[c] Approximate values for population P8 based on data from Figure 5.

[d] Populations F2EX, F1MD, P5WD and P7WD.

[e] Approximate values for population P5 based on data from Figure 5.

[f] Populations F2MD, F3MD, F4MD, P1WD, P3WD, P9WD.

Table 6
Root chemotypes in wild and cultivated populations of American ginseng (*Panax quinquefolius*) in Maryland

Population	Population chemotype[a,b]	Root Chemotype			
		High Rg1/Low Re[a]	Low Rg1/High Re[b]	Intermediate[c]	Total
Cultivated					
F2EX	Low Rg1/High Re	0	7	0	7
F1MD	Low Rg1/High Re	0	2	0	2
F2MD	High Rg1/Low Re	3	0	0	3
F3MD	High Rg1/Low Re	9	1	2	12
F4MD	High Rg1/Low Re	2	0	0	2
Wild					
P1WD	High Rg1/Low Re	2	0	0	2
P3WD	High Rg1/Low Re	6	0	0	6
P5WD	Low Rg1/High Re	0	2	1	3
P7WD	Low Rg1/High Re	0	4	0	4
P9WD	High Rg1/Low Re	2	1	0	3
Total		24	17	3	44

[a] High Rg1/Low Re chemotype (median or individual root Rg1 composition > 30%; Re composition < 20%).

[b] Low Rg1/High Re chemotype (median or individual root Rg1 composition <20%; Re composition >30%).

[c] Intermediate (individual root Rg1 composition 20–30%, Re composition > 30%).

3. Conclusions

Based on recent evidence that individual ginsenosides have divergent and important effects on health, discovering significant variation for ginsenoside content and composition and identifying two distinct American ginseng chemotypes should have significant implications for future research, development, and regulation of ginseng products. In the US, ginseng is classified as a dietary supplement and regulated by the FDA under the Dietary Supplemental Health and Education Act (DSHEA) of 1994. In April 2005, the FDA issued the 'Guidance for Industry: A Dietary Supplement Labeling Guide' to help assure that the dietary supplements sold in the US are properly labeled (FDA, 2005). The items to be contained on all labels are (1) name of the dietary supplement, (2) amount of the dietary supplement, (3) the nutrition labeling, (4) the ingredient list, and (5) the name and place of business of the manufacturer, packer, or distributor. However, these guidelines represent FDA's current opinions regarding

labels and are not legally binding. In the case of ginseng, "the term 'ginseng' may be considered to be a common or usual name (or part thereof) for any herb or herbal ingredient derived from a plant classified within the genus Panax" (FDA, 2003). Title 21 of the Code of Regulations, allows labels to omit the Latin binomial if the American Herbal Products Association (AHPA) "standardized common name" is used (Blumenthal, 2006). However, based on the differences in bioactivity between *P. ginseng* and *P. quinquefolius*, we recommend that labels containing therapeutic claims should include the Latin binomial name along with the common names of American ginseng or Asian ginseng in order to distinguish between these two species. Given the large plant to plant variation of percent total ginsenosides, we agree with the recommendation that labels of products should contain the total ginsenoside content in the supplement so that the dosage is known. Further, we believe that the substantial differences in ginsenoside composition found between ginseng populations warrants including the ginsenoside profile on the label of ginseng supplements for therapeutic usage.

4. Experimental

4.1. Plant material

In September of 2002 and 2003, the Maryland Department of Agriculture ginseng certification specialist collected 44 American ginseng roots for this study. Sixteen roots were collected from five wild populations and four Maryland growers contributed 26 roots from five cultivated field plots (Table 1). Production scale among these farms was highly variable which influenced the number of samples donated. Only two wild samples were collected in 2002 because a drought caused plants to senesce early in the growing season and plants could not be collected before August 20, the first day of legal harvest. The putative source of seed was based on the collection location (wild populations) or information supplied by the grower. Only one grower reported propagating American ginseng from non-MD seed sources (seed purchased from cultivated field plots in Tennessee or Wisconsin). Roots cultivated from these non-MD seed sources (population F2EX) provided a control group against which roots putatively grown from regional seed sources were compared.

Because root sampling for analysis was destructive and wild American ginseng is globally vulnerable and rare to uncommon in Maryland (NatureServe, 2006), we intentionally limited the number of wild roots collected from each population. Wild American ginseng is widespread but scarce everywhere it is found (McGraw et al., 2003) and the maximum sustainable harvest has been estimated at 5% based on a viability analysis of wild populations in Canada (Nantel et al., 1996). Total estimated population size for the populations surveyed in this study rarely exceeded 50 plants, thus the minimum of $n = 10$ plants per population typically suggested for statistical analyses would greatly exceed the sustainable harvest limit. Tests for significant differences were conducted including and excluding populations with a sample size of two, and the significant differences were the same for both analyses. Thus, we chose to present the complete dataset since these populations will provide baseline data for future studies.

4.2. Extraction of ginsenosides

Roots were freeze-dried for 72 h, ground in a Wiley Mill (20 mesh) (Foss Tecator AB, Sweden) and kept at room temperature until extraction. Extraction methods were based on the protocol of Li et al. (1996), but were simplified and modified for smaller samples. An accurately weighed sample (100 mg) was transferred to a 25 ml Erlenmeyer flask. Ginsenosides were extracted in MeOH–H_2O (4:1, 5 ml) in a 70 °C water bath for 1 h. During extraction, samples were stirred continuously and every 15 min flasks were removed from the water bath and vortexed briefly. Flasks were capped but vented to reduce pressure and minimize evaporation. Extracts were centrifuged for 5 min (5000 rpm, Sorvall® SA-600 rotor) (Sorvall® Inc., Newtown, CT) and filtered using a 0.45-μm filter (Fisher Scientific International Inc., Hampton, NH). Extracts were concentrated to 400 μl under a stream of N_2 and resuspended in MeOH (1.6 ml) (2 ml sample in MeOH (4:1)). Samples were re-filtered and 20 μl of extract was immediately injected in the HPLC system.

4.3. HPLC and quantification

The five most abundant ginsenosides in American ginseng roots, Rg1, Re, Rb1, Rc and Rd (Court et al., 1996a; Wills et al., 2002; Assinewe et al., 2003), were analyzed using a HP1100 high-performance liquid chromatography (HPLC) system (Agilent Technologies Inc., Palo Alto, CA) with gradient elution and a μBondapak® C18 reversed phase column (10 μm, 4.6 mm × 150 mm) with a μBondapak™ C18 Sentry™ Guard column (10 μm, 3.9 mm × 20 mm) (Waters Inc., Milford, MA). The binary gradient employed the mobile phases: (A) H_2O and (B) CH_3CN (HPLC grade; Fisher Scientific International Inc., Palo Alto, CA) with a flow rate of 1.2 ml min^{-1} according to the following profile adapted from Court et al. (1996): 0–20 min, 20–21% B; 20–25 min, 21–26% B; 25–29 min, 26–27% B; 29–43 min, 27–34%; 43–47 min, 34–36% B; 47–54 min, 36–43% B; 54–55 min, 43–95% B; 55–59 min 95% B. The UV diode array detector was set at 203 nm. Ginsenoside concentrations for each sample were calculated using respective standard curves ($r^2 > 0.99$ for each of the five ginsenosides tested) based on standards purchased from Sigma–Aldrich Fine Chemicals (Milwaukee, WI) and Indofine Chemical Company Inc. (Hillsborough, NJ). Samples were tested in duplicate. Sample order was

randomized but modified such that duplicate samples were not extracted in the same day.

4.4. Statistical analysis

Ginsenoside content was expressed as the weight of ginsenoside relative to the root dry weight (% w/w). Ginsenoside composition was expressed as the content of each ginsenoside (Rg1, Re, Rb1, Rc or Rd) relative to the total content of the five analyzed ginsenosides in that sample (% total). Contents were not considered absolute because recovery was less than 100% and not all ginsenosides were analyzed. Spearman's non-parametric rank correlations among ginsenoside variables (% w/w total ginsenoside, Rg1, Re, Rb1, Rc and Rd) were analyzed using PROC CORR in SAS (SAS Institute Inc, 2002). Ginsenoside content and composition data were not normal ($W < 0.95$, $p > 0.05$); therefore, non-parametric methods were used for statistical analyses using PROC NPAR1WAY (SAS Institute Inc, 2002). The Kruskal–Wallis test was used to determine whether there were significant differences in ginsenoside contents or compositions among the populations. Median ginsenoside contents and compositions were compared to the control population (F2EX) using the Wilcoxon rank sum test. Differences were considered statistically significant if $p < 0.05$ for the test statistic.

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References

American Botanical Council, 2002. Nature and taste in traditional Chinese medicine. HerbalGram 54, 42.

Assinewe, V.A., Baum, B.R., Gagnon, D., Arnason, J.T., 2003. Phytochemistry of wild populations of *Panax quinquefolius* L. (North American ginseng). J. Agric. Food Chem. 51, 4549–4553.

Attele, A.S., Wu, J.A., Yuan, C.-S., 1999. Ginseng pharmacology: multiple constituents and multiple actions. Biochem. Pharmacol. 58, 1685–1693.

Awang, D.V.C., 2000. The neglected ginsenosides of North American ginseng (*Panax quinquefolius* L.). J Herbs Spices Med Plants 7, 103–109.

Beyfuss, R.L., 1999. Economics and Marketing of Ginseng. Agroforestry Notes, USFS. AF Note: 14, pp. 1–4.

Blumenthal, M., 2003. International trade centre market news service launches reports on worldwide herb markets. HerbalGram 57, 57–59.

Blumenthal, M., 2006. Herb industry self-regulation initiative on common names for herbal ingredients becomes federal law in January. HerbalGram 69, 60–61.

Chamberlain, J.L., Predny, M., 2005. Market economics of ginseng. Encyclopedia ID: 632. Available at: http://www.forestryencyclopedia.net (accessed February 10, 2005).

Chuang, W.-C., Wu, H.-K., Sheu, S.-J., Chiou, S.-H., Chang, H.-C., Chen, Y.-P., 1995. A comparative study of commercial samples of ginseng radix. Planta Med. 61, 459–465.

Court, W.A., Hendel, J.G., Elmi, J., 1996a. Reversed-phase high-performance liquid chromatography determination of ginsenosides of *Panax quinquefolium*. J. Chromatogr. A 755, 11–17.

Court, W.A., Reynolds, L.B., Hendel, J.G., 1996b. Influence of root age on the concentration of ginsenosides of American ginseng (*Panax quinquefolium*). Can. J. Plant Sci. 76, 853–855.

Cruse-Sanders, J.M., Hamrick, J.L., 2004. Genetic diversity in harvested and protected populations of wild American ginseng, *Panax quinquefolius* L. (Araliaceae). Am. J. Bot. 91, 540–548.

Dharmananda, S., 2002. The nature of ginseng: traditional use, modern research, and the question of dosage. HerbalGram 54, 34–51.

FDA, 2003. Food labeling: ingredient labeling of dietary supplements that contain botanicals. Docket No. 2003N-0346 (Food and Drug Administration). Fed. Register 68 (167), 51738–51749. Available from: http://www.ahpa.org/03_0828_DirectFinalRule_HoC2.pdf .

FDA, 2005. Guidance for Industry: The Dietary Supplement Labeling Guide. Available at: http://www.cfsan.fda.gov/~dms/dslg_toc.html#toc (accessed May 5, 2005).

Fournier, A.R., Proctor, J.T.A., Gauthier, L., Khanizadeh, S., Belanger, A., Gosselin, A., Dorais, M., 2003. Understory light and root ginsenosides in forest-grown *Panax quinquefolius*. Phytochemistry 63, 777–782.

Grubbs, H.J., Case, M.A., 2004. Allozyme variation in American ginseng (*Panax quinquefolius* L.): variation, breeding system, and implications for current conservation practice. Conserv. Genet. 5, 13–23.

Hall, T., Lu, Z.-z., Yat, P.N., Fitzloff, J.F., Arnason, J.T., Awang, D.V.C., Fong, H.H.S., Blumenthal, M., 2001. Evaluation of consistency of standardized Asian ginseng products in the ginseng evaluation program. HerbalGram 52, 31–54.

Li, T.S.C., Mazza, G., 1999. Correlations between leaf and soil mineral concentrations and ginsenoside contents in American ginseng. Hortscience 34, 85–87.

Li, T.S.C., Mazza, G., Cottrell, A.C., Gao, L., 1996. Ginsenosides in roots and leaves of American ginseng. J. Agric. Food Chem. 44, 717–720.

Lim, W., Mudge, K.W., Vermeylen, F., 2005. Effects of population, age, and cultivation methods on ginsenoside content of wild American ginseng (*Panax quinquefolium*). J. Agric. Food Chem. 53, 8498–8505.

McGraw, J.B., Sanders, S.M., Van der Voort, M., 2003. Distribution and abundance of *Hydrastis canadensis* L. (Ranunculaceae) and *Panax quinquefolius* L. (Araliaceae) in the central Appalachian region. J. Torrey Bot. Soc. 130, 62–69.

Nantel, P., Gagnon, D., Nault, A., 1996. Population viability analysis of American ginseng and wild leek harvested in stochastic environments. Conserv. Biol. 10, 608–621.

NatureServe. 2006. NatureServe Explorer: An Online Encyclopedia of Life [web application]. Version 4.7. NatureServe, Arlington, Virginia. Available at: http://www.natureserve.org/explorer (accessed April 10, 2006).

Robbins, C.S., 2000. Comparative analysis of management regimes and medicinal plant trade monitoring mechanisms for American ginseng and goldenseal. Conserv. Biol. 14, 1422–1434.

SAS Institute Inc., 2002. The SAS System for Windows®, Version 8.2. Cary, NC, USA.

Sengupta, S., Toh, S.-A., Sellers, L.A., Skepper, J.N., Koolwijk, P., Leung, H.W., Yeung, H.-W., Wong, R.N.S., Sasisekharan, R., Fan, T.-P.D., 2004. Modulating angiogenesis: the yin and the yang in ginseng. Circulation 110, 1219–1225.

Shibata, S., 2001. Chemistry and cancer preventing activities of ginseng saponins and some related triterpenoid compounds. J. Korean Med. Sci. 16 (Suppl.), S28–S37.

Sievenpiper, J.L., Arnason, J.T., Leiter, L.A., Vuksan, V., 2004. Decreasing, null and increasing effects of eight popular types of ginseng on acute postprandial glycemic indices in healthy humans: the role of ginsenosides. J. Am. Coll. Nutr. 23, 248–258.

Smith, R.G., Caswell, D., Carriere, A., Zielke, B., 1996. Variation in the ginsenoside content of American ginseng, *Panax quinquefolius* L. roots. Can. J. Bot. 74, 1616–1620.

Wang, A., Wang, C.-Z., Wu, J.-A., Osinski, J., Yuan, C.-S., 2005. Determination of major ginsenosides in *Panax quinquefolius* (American ginseng) using high-performance liquid chromatography. Phytochem. Anal. 16, 272–277.

Wills, R.B.H., Du, X.W., Stuart, D.I., 2002. Changes in ginsenosides in Australian-grown American ginseng plants (*Panax quinquefolium* L.). Aust. J. Exp. Agric. 42, 1119–1123.